Access DB# 37309

# SEARCH REQUEST FORM

MEJ

**Scientific and Technical Information Center**

Requester's Full Name: Terri McCaa Examiner #: 75017 Date: 09 Mar 01
Art Unit: 1641 Phone Number 30 605 1207 Serial Number: 09/716,554
Mail Box and Bldg/Room Location: 7D06 Results Format Preferred (circle): PAPER DISK E-MAIL

TER

**If more than one search is submitted, please prioritize searches in order of need.**

**********

Please provide a detailed statement of the search topic, and describe as specifically as possible the subject matter to be searched. Include the elected species or structures, keywords, synonyms, acronyms, and registry numbers, and combine with the concept or utility of the invention. Define any terms that may have a special meaning. Give examples or relevant citations, authors, etc, if known. Please attach a copy of the cover sheet, pertinent claims, and abstract.

Title of Invention: Bifunctional molecules and their use in the disruption of protein-protein interactions

Inventors (please provide full names): Gerald M. Rubanyi, Eiji Shinkawa, Ryan Briesewitz, Thomas T. Daniels

Earliest Priority Filing Date: 11/17/2000

***For Sequence Searches Only* Please include all pertinent information (parent, child, divisional, or issued patent numbers) along with the appropriate serial number.**

Synthetic bifunctional inhibitor molecule
less than 5000 daltons

Inhibitor consist of Protein ligand; blocking protein ligand joined by a linker

Blocking protein is peptidyl prolyl isomerase
" " intracellular protein
" " extracellular protein

Point of Contact:
Beverly Shears
Technical Info. Specialist
CM1 12C14 Tel: 306-4954

**********

| STAFF USE ONLY | Type of Search | | Vendors and cost where applicable |
|---|---|---|---|
| Searcher: Beverly 4954 | NA Sequence (#) | | STN |
| Searcher Phone #: | AA Sequence (#) | | Dialog |
| Searcher Location: | Structure (#) | | Questel/Orbit |
| Date Searcher Picked Up: | Bibliographic | | Dr.Link |
| Date Completed: 03-16-01 | Litigation | | Lexis/Nexis |
| Searcher Prep & Review Time: 12 | Fulltext | | Sequence Systems |
| Clerical Prep Time: | Patent Family | | WWW/Internet |
| Online Time: 24 | Other | | Other (specify) |

PTO-1590 (1-2000)

McCaa
09/716054



(FILE 'REGISTRY' ENTERED AT 09:30:47 ON 16 MAR 2001)

L1 5 SEA FILE=REGISTRY ABB=ON PLU=ON PEPTIDYLPROLYL ISOMERASE ?/CN

L15 1 SEA FILE=REGISTRY ABB=ON PLU=ON "ISOMERASE, PEPTIDYL-PROLYL CIS-TRANS- (TOLYPOCLADIUM NIVEUM CLONE PSIM10 GENE CPTA)"/CN

L16 230 SEA FILE=REGISTRY ABB=ON PLU=ON ISOMERASE, PEPTIDYLPROLYL ?/CN

L17 1 SEA FILE=REGISTRY ABB=ON PLU=ON "PEPTIDYL PROLYL-CIS-TRANS ISOMERASE-HOMOLOGOUS (SOYBEAN)"/CN

L18 3 SEA FILE=REGISTRY ABB=ON PLU=ON ("PEPTIDYL PROLYL CIS-TRANS ISOMERASE (METHANOCOCCUS THERMOLITHOTROPHICUS STRAIN DSM 2095 GENE MTFK)"/CN OR "PEPTIDYL PROLYL CIS/TRANS ISOMERASE (ARABIDOPSIS THALIANA CLONE PIN1ATPET 19B GENE PIN1AT)"/CN OR "PEPTIDYL PROLYL-CIS-TRANS ISOMERASE-HOMOLOGOUS (SOYBEAN)"/CN)

L19 231 SEA FILE=REGISTRY ABB=ON PLU=ON L1 OR L15 OR L16 OR L17 OR L18

(FILE 'CAPLUS' ENTERED AT 09:32:17 ON 16 MAR 2001)

L1 5 SEA FILE=REGISTRY ABB=ON PLU=ON PEPTIDYLPROLYL ISOMERASE ?/CN

L15 1 SEA FILE=REGISTRY ABB=ON PLU=ON "ISOMERASE, PEPTIDYL-PROLYL CIS-TRANS- (TOLYPOCLADIUM NIVEUM CLONE PSIM10 GENE CPTA)"/CN

L16 230 SEA FILE=REGISTRY ABB=ON PLU=ON ISOMERASE, PEPTIDYLPROLYL ?/CN

L17 1 SEA FILE=REGISTRY ABB=ON PLU=ON "PEPTIDYL PROLYL-CIS-TRANS ISOMERASE-HOMOLOGOUS (SOYBEAN)"/CN

L18 3 SEA FILE=REGISTRY ABB=ON PLU=ON ("PEPTIDYL PROLYL CIS-TRANS ISOMERASE (METHANOCOCCUS THERMOLITHOTROPHICUS STRAIN DSM 2095 GENE MTFK)"/CN OR "PEPTIDYL PROLYL CIS/TRANS ISOMERASE (ARABIDOPSIS THALIANA CLONE PIN1ATPET 19B GENE PIN1AT)"/CN OR "PEPTIDYL PROLYL-CIS-TRANS ISOMERASE-HOMOLOGOUS (SOYBEAN)"/CN)

L19 231 SEA FILE=REGISTRY ABB=ON PLU=ON L1 OR L15 OR L16 OR L17 OR L18

L20 4580 SEA FILE=CAPLUS ABB=ON PLU=ON L19 OR (BLOCK? OR INTRACELL? OR INTRA CELL?)(W)PROTEIN OR (PEPTIDYLPROLYL OR PEPTIDYL PROLYL)(3W)ISOMERASE

L21 13 SEA FILE=CAPLUS ABB=ON PLU=ON L20 AND (BIFUNCT? OR BIFUNCT?)

L1 5 SEA FILE=REGISTRY ABB=ON PLU=ON PEPTIDYLPROLYL ISOMERASE ?/CN

L15 1 SEA FILE=REGISTRY ABB=ON PLU=ON "ISOMERASE, PEPTIDYL-PROLYL CIS-TRANS- (TOLYPOCLADIUM NIVEUM CLONE PSIM10 GENE CPTA)"/CN

Searcher : Shears 308-4994

L16 230 SEA FILE=REGISTRY ABB=ON PLU=ON ISOMERASE, PEPTIDYLPROL YL ?/CN
L17 1 SEA FILE=REGISTRY ABB=ON PLU=ON "PEPTIDYL PROLYL-CIS-TR ANS ISOMERASE-HOMOLOGOUS (SOYBEAN)"/CN
L18 3 SEA FILE=REGISTRY ABB=ON PLU=ON ("PEPTIDYL PROLYL CIS-TRANS ISOMERASE (METHANOCOCCUS THERMOLITHOTROPHICUS STRAIN DSM 2095 GENE MTFK)"/CN OR "PEPTIDYL PROLYL CIS/TRANS ISOMERASE (ARABIDOPSIS THALIANA CLONE PIN1ATPET 19B GENE PIN1AT)"/CN OR "PEPTIDYL PROLYL-CIS-TRANS ISOMERASE-HOMOLOGOUS (SOYBEAN)"/CN)
L19 231 SEA FILE=REGISTRY ABB=ON PLU=ON L1 OR L15 OR L16 OR L17 OR L18
L20 4580 SEA FILE=CAPLUS ABB=ON PLU=ON L19 OR (BLOCK? OR INTRACELL? OR INTRA CELL?)(W)PROTEIN OR (PEPTIDYLPROLYL OR PEPTIDYL PROLYL)(3W)ISOMERASE
L22 752 SEA FILE=CAPLUS ABB=ON PLU=ON L20 AND INHIBITOR
L23 73 SEA FILE=CAPLUS ABB=ON PLU=ON L22 AND (CONJUGAT? OR LINK?)
L24 12 SEA FILE=CAPLUS ABB=ON PLU=ON L23 AND LIGAND

L25 24 L21 OR L24

=> d 1-24 .bevstr

L25 ANSWER 1 OF 24 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:816385 CAPLUS
DOCUMENT NUMBER: 134:145106
TITLE: Proteasome **inhibitor**-induced apoptosis of glioma cells involves the processing of multiple caspases and cytochrome c release
AUTHOR(S): Wagenknecht, Bettina; Hermisson, Mirjam; Groscurth, Peter; Liston, Peter; Krammer, Peter H.; Weller, Michael
CORPORATE SOURCE: Laboratory of Molecular Neuro-Oncology, Department of Neurology, School of Medicine, University of Tubingen, Tubingen, 72076, Germany
SOURCE: J. Neurochem. (2000), 75(6), 2288-2297
CODEN: JONRA9; ISSN: 0022-3042
PUBLISHER: Lippincott Williams & Wilkins
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The proteasome is a multiprotein complex that is involved in the **intracellular protein** degrdn. in eukaryotes. Here, we show that human malignant glioma cells are susceptible to apoptotic cell death induced by the proteasome **inhibitors**, MG132 and lactacystin. The execution of the apoptotic death program involves the processing of caspases 2, 3, 7, 8, and 9. Apoptosis is inhibited by ectopic expression of X-**linked** **inhibit r** of apoptosis (XIAP) and by co-exposure to the

broad-spectrum caspase **inhibitor**, benzoyl-VAD-fluoromethyl ketone (zVAD-fmk), but not by the preferential caspase 8 **inhibitor**, crm-A. It is interesting that specific morphol. alterations induced by proteasome inhibition, such as dilated rough endoplasmic reticulum and the formation of cytoplasmic vacuoles and dense mitochondrial deposits, are unaffected by zVAD-fmk. Apoptosis is also inhibited by ectopic expression of Bcl-2 or by an **inhibitor** of protein synthesis, cycloheximide. Further, cytochrome c release and disruption of mitochondrial membrane potential are prominent features of apoptosis triggered by proteasome inhibition. Bcl-2 is a stronger **inhibitor** of cytochrome c release than zVAD-fmk. XIAP and crm-A fail to modulate cytochrome c release. These data place cytochrome c release downstream of Bcl-2 activity but upstream of XIAP- and crm-A-sensitive caspases. The partial inhibition of cytochrome c release by zVAD-fmk indicates a pos. feedback loop that may involve cytochrome c release and zVAD-fmk-sensitive caspases. Finally, death **ligand**/receptor interactions, including the CD95/CD95 **ligand** system, do not mediate apoptosis induced by proteasome inhibition in human malignant glioma cells.

REFERENCE COUNT: 27
REFERENCE(S):
(1) Chang, Y; Cell Growth Differ 1998, V9, P79 CAPLUS
(2) Chinnaiyan, A; J Biol Chem 1996, V271, P4573 CAPLUS
(3) Coux, O; Annu Rev Biochem 1996, V65, P801 CAPLUS
(5) Drexler, H; Proc Natl Acad Sci USA 1997, V94, P855 CAPLUS
(6) Fearnhead, H; Proc Natl Acad Sci USA 1998, V95, P13664 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L25 ANSWER 2 OF 24 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:165842 CAPLUS
DOCUMENT NUMBER: 133:87727
TITLE: Reduced aldehyde dehydrogenase levels in the brain of patients with Down syndrome
AUTHOR(S): Lubec, G.; Labudova, O.; Cairns, N.; Berndt, P.; Langen, H.; Fountoulakis, M.
CORPORATE SOURCE: Department of Pediatrics, University of Vienna, Vienna, Austria
SOURCE: J. Neural Transm., Suppl. (1999), 57(Molecular Biology of Down Syndrome), 21-40
CODEN: JNTSD4; ISSN: 0303-6995
PUBLISHER: Springer-Verlag Wien
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Aldehyde dehydrogenase (ALDH) is a key enzyme in fructose,

acetaldehyde and oxalate metab. and represents a major detoxification system for reactive carbonyls and aldehydes. In the brain, ALDH exerts a major function in the metab. of biogenic aldehydes, norepinephrine, dopamine and diamines and .gamma.-aminobutyric acid. Subtractive hybridization studies in Down Syndrome (DS) fetal brain showed that mRNA for ALDH are downregulated. Here we studied the protein levels in the brain of adult patients. The proteins from five brain regions of 9 aged patients with DS and 9 controls were analyzed by two-dimensional (2-D) gel electrophoresis and identified by matrix-assisted laser desorption ionization mass spectrometry. ALDH levels were reduced in the brain regions of at least half of the patients with Down Syndrome, as compared to controls. The decreased ALDH levels in the DS brain may result in accumulation of aldehydes which can lead to the formation of plaques and tangles reflecting abnormally cross-**linked**, insol. and modified proteins, found in aged DS brain. Furthermore, we constructed a 2-D map including approx. 120 identified human brain proteins.

IT **95076-93-0**
RL: BOC (Biological occurrence); BIOL (Biological study); OCCU (Occurrence)
(aldehyde dehydrogenase and various proteins in human brain regions of patients with Down syndrome)

REFERENCE COUNT: 35
REFERENCE(S):
(1) Bradford, M; Anal Biochem 1976, V72, P248 CAPLUS
(2) Busciglio, J; Nature 1995, V378, P776 CAPLUS
(3) Colzi, A; J Neurochem 1996, V66, P1510 CAPLUS
(5) Esterbauer, H; Free Radic Biol Med 1991, V11, P81 CAPLUS
(6) Fountoulakis, M; Anal Biochem 1997, V250, P153 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L25 ANSWER 3 OF 24 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:763899 CAPLUS
DOCUMENT NUMBER: 132:15629
TITLE: **Bifunctional** molecules and therapies based thereon
INVENTOR(S): Briesewitz, Roger; Crabtree, Gerald R.; Wandless, Thomas; Ray, Gregory Thomas; Vogel, Kurt William
PATENT ASSIGNEE(S): The Board of Trustees of the Leland Stanford Junior University, USA
SOURCE: PCT Int. Appl., 67 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
Searcher : Shears 308-4994

FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 9961055 | A1 | 19991202 | WO 1999-US11296 | 19990521 |
| W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | | |
| AU 9940937 | A1 | 19991213 | AU 1999-40937 | 19990521 |
| EP 1079859 | A1 | 20010307 | EP 1999-924431 | 19990521 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI | | | | |
| PRIORITY APPLN. INFO.: | | | US 1998-86451 | 19980522 |
| | | | WO 1999-US11296 | 19990521 |

OTHER SOURCE(S): MARPAT 132:15629

AB Non-naturally occurring **bifunctional conjugates** Z-L-X (Z = **ligand** that binds to a specific presenter protein; X = drug moiety; L = optional **linker**) are provided such that upon entering a cell, Z can bind to its receptor protein (if present) and the effectiveness of X is thereby enhanced or inhibited, depending on the nature of the receptor for Z. Thus, a **bifunctional** peptide (I) was prepd. which contained FK506 coupled to phosphotyrosyl-glutamyl-glutamyl-isoleucine (pYEEI), which binds to the SH2 domains of tyrosine kinases Fyn and Lck and to the N-terminal SH2 domain of phospholipase C.gamma. (PLC.gamma.). In the presence of FK506-binding protein 52 (FKBP52), I bound the Fyn SH2 domain with 3-fold increased affinity. This effect was reversed by FK506, and was not mimicked by FKBP12 despite the similar structure of its binding domain to that of FKBP52; the increase in affinity with FKBP52 was presumably based on favorable protein-protein interactions between the Fyn SH2 domain and FKBP52. On the other hand, formation of a FKBP12-I complex reduced the affinity of I for the PLC.gamma. SH2 domain but not for the Fyn or Lek SH2 domains, suggesting that formation of a binary complex may lead to unfavorable protein-protein interactions between the presenter protein and some targets but not other targets of the drug; therefore, formation of a complex between a **bifunctional** mol. and a presenter protein can be used to create specificity. The cell selectivity of a **bifunctional conjugate** may be enhanced if the formation of a binary complex reduces binding of the drug to all of its targets in a cell that contains the presenter mol.; if an organism has cells that

contain the presenter protein and other cells that do not, the cells lacking the presenter protein will be more affected by the **bifunctional conjugate** than cells expressing the presenter. Similarly, **conjugation** of penicillamine (an alk. phosphatase **inhibitor**) to p-aminosalicylic acid (a **ligand** for albumin) via glycine modulated the inhibitory activity of penicillamine toward 4 isoforms of alk. phosphatase in the presence of 100 .mu.M serum albumin, but not toward 8 other isoforms.

IT **95076-93-0, Peptidyl prolyl isomerase**
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(binding by protein **ligand**-drug **bifunctional conjugates**; **bifunctional** mols. and therapies based thereon)

REFERENCE COUNT: 21
REFERENCE(S):
(1) Al-Obeidi; Journal of Pharmaceutical Sciences 1990, V79(6), P500 CAPLUS
(2) Belshaw; Proc Natl Acad Sci USA 1996, V93, P4604 CAPLUS
(3) Briesewitz; Proc Natl Acad Sci USA 1999, V96(5), P1953 CAPLUS
(4) Brochu; Antimicrobial Agents and Chemotherapy 1992, V36(10), P2166 CAPLUS
(5) Cemu Bioteknik Ab; WO 9101743 A1 1991 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L25 ANSWER 4 OF 24 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:748231 CAPLUS
DOCUMENT NUMBER: 132:421
TITLE: Use of nuclear magnetic resonance to design **ligands** to target biomolecules
INVENTOR(S): Fesik, Stephen W.; Hajduk, Philip J.; Olejniczak, Edward T.
PATENT ASSIGNEE(S): Abbott Laboratories, USA
SOURCE: U.S., 35 pp., Cont.-in-part of U.S. Ser. No. 678,903, abandoned.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| US 5989827 | A | 19991123 | US 1996-744701 | 19961031 |
| US 5891643 | A | 19990406 | US 1995-558633 | 19951114 |

PRIORITY APPLN. INFO.: US 1995-558633 19951114

Searcher : Shears 308-4994

US 1996-678903 19960712

AB The present invention provides a method for the use of two-dimensional 15N/1H NMR correlation spectral anal. to design **ligands** that bind to target biomols., such as proteins. The process includes the steps of (a) identifying a first and a second **ligand** to the target mol. using two-dimensional 15N/1H NMR correlation spectroscopy; (b) forming a ternary complex by binding the first and second **ligands** to the target mol.; (c) detg. the three dimensional structure of the ternary complex and thus the spatial orientation of the first and second **ligands** on the target mol.; and (d) **linking** the first and second **ligands** to form the drug, wherein the spatial orientation of step (c) is maintained. E.g., protein solns. contg. the uniformly 15N-labeled catalytic domain of human stromelysin (fragment 81-256) were prepd. and used for screening of a database of test compds. by a two-dimensional 15N/1H NMR correlation spectral anal. In the initial screen, two compds. contg. a biaryl moiety were found that bind to the catalytic domain of stromelysin. Based on these initial hits, structurally similar compds. were tested against stromelysin. In the sec. round of screening, binding was assayed both in the absence and the presence of satg. amts. of acetohydroxamic acid (as the first **ligand**). The ability to locate the specific binding site of a particular **ligand** is an advantage of the present invention.

IT **131144-19-9**
RL: PRP (Properties)
(unclaimed protein sequence; use of NMR to design **ligands** to target biomols.)

REFERENCE COUNT: 23
REFERENCE(S):
(1) Anon; WO 9110140 1991 CAPLUS
(2) Anon; WO 9300446 1993 CAPLUS
(3) Anon; WO 9414980 1994 CAPLUS
(4) Anon; WO 9418339 1994 CAPLUS
(5) Bax, A; Accounts of Chemical Research 1993, V26(4), P131 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L25 ANSWER 5 OF 24 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:610244 CAPLUS
DOCUMENT NUMBER: 132:45357
TITLE: Pharmacological inhibition of Ras-transformed epithelial cell growth is **linked** to down-regulation of epidermal growth factor-related peptides
AUTHOR(S): Sizemore, Nywana; Cox, Adrienne D.; Barnard, John A.; Oldham, Sean M.; Reynolds, Evangeline R.; Der, Channing J.; Coffey, Robert J.
CORPORATE SOURCE: Departments of Medicine and Cell Biology, Vanderbilt University and Veterans Affairs

Medical Center, Nashville, TN, USA
SOURCE: Gastroenterology (1999), 117(3), 567-576
CODEN: GASTAB; ISSN: 0016-5085
PUBLISHER: W. B. Saunders Co.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Posttranslational farnesylation is required for Ras activation. Farnesyltransferase **inhibitors** (FTIs) selectively **block protein** farnesylation and reduce the growth of many Ras-transformed cells in vitro and in vivo. Activated Ras transforms rat intestinal epithelial (RIE-1) cells by a mechanism distinct from NIH 3T3 fibroblasts in that an epidermal growth factor receptor (EGFR) autocrine loop contributes significantly to the Ras-transformed RIE-1 phenotype. The ability of FTIs to block growth of Ras-transformed RIE-1 cells was evaluated, and these results were correlated with decreased EGFR **ligand** prodn. FTI L744,832 caused a selective, dose-dependent, reversible blockade in proliferation of H-Ras-transformed RIE-1 cells, whereas control cell lines, K-Ras-transformed cells, and activated raf-transfected RIE cells were unaffected. The growth-inhibitory effects of L744,832 correlated with loss of farnesylated H-Ras protein and a marked redn. in transforming growth factor (TGF)-.alpha. and amphiregulin expression. Inhibition of proliferation of H-Ras RIE-1 cells by L744,832 was overcome by exogenous TGF-.alpha., and enhanced growth inhibition was achieved by EGFR blockade in combination with L744,832. These data suggest that one mechanism by which FTIs inhibit growth of H-Ras-transformed epithelial cells is by reducing Ras-induced EGFR **ligand** prodn.

REFERENCE COUNT: 49
REFERENCE(S):
(1) Barnard, J; J Biol Chem 1994, V269, P22817 CAPLUS
(2) Barnard, J; Proc Natl Acad Sci USA 1989, V86, P1578 CAPLUS
(3) Blay, J; Biochem J 1985, V225, P85 CAPLUS
(5) Bos, J; Cancer Res 1989, V49, P4682 CAPLUS
(7) Coffey, R; Cancer Res 1988, V48, P1596 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L25 ANSWER 6 OF 24 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:92751 CAPLUS
TITLE: Synthesis of thioester FKBP12 **ligands** and evaluation of their in vitro and in vivo nerve regenerative effects
AUTHOR(S): Limburg, David C.; Vaal, Mark J.; Li, Jia-He; Wu, Yong-Qian; Thomas, Christine; Sauer, Hansjorg; Ross, Douglas T.; Soni, Rajit; Chen, Yi; Guo, Hongshi; Howorth, Pamela; Valentine, Heather; Liang, Shi; Spicer, Dawn; Fuller, Mike;

Steiner, Joseph P.; Hamilton, Gregory S.
CORPORATE SOURCE: Guilford Pharmaceuticals, Inc., Baltimore, MD, 21224, USA
SOURCE: Book of Abstracts, 217th ACS National Meeting, Anaheim, Calif., March 21-25 (1999), MEDI-237. American Chemical Society: Washington, D. C.
CODEN: 67GHA6
DOCUMENT TYPE: Conference; Meeting Abstract
LANGUAGE: English
AB **Ligands** for the **peptidyl-prolyl isomerase** FKBP12 have been found to unexpectedly possess powerful neuroprotective and neuroregenerative effects in vitro and in vivo. We have extensively explored the therapeutic utility of FKBP12 **ligands** based on esters of proline and pipecolic acid. Here we describe a new class of FKBP12 **ligand** contg. a thioester **linkage**. These novel FKBP12 **ligands** are effective in a rodent model of Parkinson's Disease following either systemic or oral administration. Details of the in vitro SAR of these compds. as FKBP12 **inhibitors**, and their in vivo efficacy as neuroregenerative agents, will be discussed.

L25 ANSWER 7 OF 24 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1998:658855 CAPLUS
DOCUMENT NUMBER: 130:24084
TITLE: The regulation of CD95 **ligand** expression and function in CTL
AUTHOR(S): Li, Jie-hui; Rosen, Dalia; Ronen, Denise; Behrens, Christian K.; Krammer, Peter H.; Clark, William R.; Berke, Gideon
CORPORATE SOURCE: Dept. Immunology, Weizmann Inst. Sci., Rehovot, 76100, Israel
SOURCE: J. Immunol. (1998), 161(8), 3943-3949
CODEN: JOIMA3; ISSN: 0022-1767
PUBLISHER: American Association of Immunologists
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Previous studies with CTL lines and CTL hybridomas have suggested that functional CD95 (APO-1/Fas)-**ligand** (CD95L) expression on effector CTLs is a consequence of specific CTL-target recognition and TCR triggering of newly transcribed CD95L. Such a control on the expression of CD95L could provide a double safeguard for killing only cognate target cells. Here the regulation of CD95L expression and function was tested in in vivo primed, alloreactive peritoneal exudate CTL (PEL) from perforin-deficient (P0) mice. CD95L-based, PEL-mediated cytotoxicity was blocked by brefeldin A, an **inhibitor** of **intracellular protein** transport, but not by the protein synthesis **inhibitor** emetine, the immunosuppressive drug cyclosporin A, or the DNA

transcription **inhibitor** actinomycin D. CD95L mRNA transcripts in freshly isolated PEL were shown by RT-PCR; CD95L surface expression was evident by staining with Fas-Fc as well as CD95L Abs. Undiminished CD95L expression and cytocidal activity were found in PEL incubated for 48 h in culture, without adding antigen, mitogen, or cytokines. PEL expressed functional CD95L, yet exhibited target cell-specific killing, except when encountering high CD95-expressing cells. Thus, PEL use CD95L probably expressed in the Golgi and/or on the cell surface and do not require newly transcribed CD95L upon target cell **conjugation**. Hence the TCR-triggered recruitment of preformed CD95L, rather than its biosynthesis, controls CD95L-based specific lysis induced by CTL.

REFERENCE COUNT: 51

REFERENCE(S):
(1) Anel, A; Eur J Immunol 1994, V24, P2469 CAPLUS
(2) Berke, G; Annu Rev Immunol 1994, V12, P735 CAPLUS
(3) Berke, G; Cell 1995, V81, P9 CAPLUS
(4) Berke, G; Immunol Today 1991, V12, P396 CAPLUS
(5) Berke, G; Immunology 1993, V78, P105 CAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L25 ANSWER 8 OF 24 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1998:530798 CAPLUS

TITLE: Terpyridyl derivatives as **bifunctional** chelates

AUTHOR(S): Putnam, William C.; Sampath, UmaShanker; Osiek, Todd A.; Touami, Sofia; Xie, Jin; Cohen, Daniel; Bashkin, James K.

CORPORATE SOURCE: Department Chemistry, Washington University, St. Louis, MO, 63130, USA

SOURCE: Book of Abstracts, 216th ACS National Meeting, Boston, August 23-27 (1998), ORGN-436. American Chemical Society: Washington, D. C.
CODEN: 66KYA2

DOCUMENT TYPE: Conference; Meeting Abstract

LANGUAGE: English

AB Metal complexes have found applications in the development of diagnostic and therapeutic pharmaceuticals and for investigating DNA and RNA structures, cleavage, and repair. A current focus in the development of diagnostic and therapeutic pharmaceuticals involves the use of **bifunctional** chelates which are capable of simultaneously coordinating to a metal and covalently bonding to a biol. carrier for targeting specific diseases. A no. of 2,2':6',2"-terpyridine (trpy) derivs. modified at the 4' position have been synthesized by three different techniques which allow them to behave as **bifunctional** chelates. The target derivs. have amino, hydroxy, halo and carboxylate groups pendant to the 4'

position to allow attachment to a biotargeting moiety. These reagents are useful for incorporation of the trpy ligand and its complexes into many substrates, including DNA building **blocks**, **proteins**, and surfaces.

L25 ANSWER 9 OF 24 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1998:452903 CAPLUS
DOCUMENT NUMBER: 129:187355
TITLE: Role for tyrosine phosphorylation and Lyn tyrosine kinase in Fas receptor-mediated apoptosis in eosinophils
AUTHOR(S): Simon, Hans-Uwe; Yousefi, Shida; Dibbert, Birgit; Hebestreit, Holger; Weber, Martina; Branch, Donald R.; Blaser, Kurt; Levi-Schaffer, Francesca; Anderson, Gary P.
CORPORATE SOURCE: Swiss Institute of Allergy and Asthma Research (SIAF), University of Zurich, Davos, CH-7270, Switz.
SOURCE: Blood (1998), 92(2), 547-557
CODEN: BLOOAW; ISSN: 0006-4971
PUBLISHER: W. B. Saunders Co.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Fas **ligand**/Fas receptor mol. interactions have been implicated as having an important function for the regulation of eosinophil apoptosis. The purpose of the present study was to investigate biochem. events triggered by the engagement of the Fas receptor in freshly isolated human and mouse eosinophils. Activation of the Fas receptor on eosinophils with the agonistic anti-Fas monoclonal antibody (MoAb) resulted in increased tyrosine phosphorylation of several **intracellular proteins** . The tyrosine kinase **inhibitors** lavendustin A and genistein inhibited Fas receptor-induced cell death in both human and mouse eosinophils in vitro and prevented, at least partially, Fas receptor-mediated resoln. of eosinophilic inflammation in a mouse in vivo model of lung eosinophilia. In addn., in freshly purified human eosinophils, lavendustin A prevented anti-Fas MoAb-induced proteolytic cleavage of lamin B, suggesting that tyrosine kinases may amplify the proteolytic signaling cascade within interleukin-1.beta. converting enzyme (ICE) family proteases. Moreover, the tyrosine kinase Lyn was identified as being involved in Fas receptor-mediated cell death. Collectively, these results demonstrate that tyrosine phosphorylation is an important step in the generation of the Fas receptor-**linked** transmembrane death signal in eosinophils and that Lyn participates in this pathway.

L25 ANSWER 10 OF 24 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1998:209149 CAPLUS

DOCUMENT NUMBER: 129:3144
TITLE: The cleaved peptide of the thrombin receptor is a strong platelet agonist
AUTHOR(S): Furman, Mark I.; Liu, Longbin; Benoit, Stephen E.; Becker, Richard C.; Barnard, Marc R.; Michelson, Alan D.
CORPORATE SOURCE: Cent. Platelet Function Studies, Univ. Massachusetts Med. Cent., Worcester, MA, 01655, USA
SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1998), 95(6), 3082-3087
CODEN: PNASA6; ISSN: 0027-8424
PUBLISHER: National Academy of Sciences
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Thrombin cleaves its G-protein-**linked** seven-transmembrane domain receptor, thereby releasing a 41-aa peptide and generating a new amino terminus that acts as a tethered **ligand** for the receptor. Peptides corresponding to the new amino terminal end of the proteolyzed seven-transmembrane domain thrombin receptor [TR42-55, SFLLRNPNDKYEPF, also known as TRAP (thrombin receptor-activating peptide)], previously have been demonstrated to activate the receptor. In this study, the authors demonstrate that the 41-aa cleaved peptide, TR1-41 (MGPRRLLLVAACFSLCGPLLSARTRARRPESKA TNATLDPR) is a strong platelet agonist. TR1-41 induces platelet aggregation. In whole-blood flow cytometric studies, TR1-41 was shown to be more potent than TR42-55 and almost as potent as thrombin, as detd. by the degree of increase in: (i) platelet surface expression of P-selectin (reflecting .alpha. granule secretion); (ii) exposure of the fibrinogen binding site on the glycoprotein (GP) IIb-IIIa complex; and (iii) fibrinogen binding to the activated GPIIb-IIIa complex. As detd. by expts. with **inhibitors** [prostaglandin I2, staurosporine, wortmannin, the endothelium-derived relaxing factor congener S-nitroso-N-acetylcysteine (SNAC), EDTA, EGTA, and genestein], and with Bernard-Soulier or Glanzmann's platelets, the authors demonstrated that TR1-41-induced platelet activation is: (i) inhibited by cAMP; (ii) mediated by protein kinase C, phosphatidyl inositol-3-kinase, myosin light chain kinase, and **intracellular protein** tyrosine kinases; (iii) dependent on extracellular calcium; and (i.v.) independent of the GPIb-IX and GPIIb-IIIa complexes. TR1-41-induced platelet activation was synergistic with TR42-55. In summary, the cleaved peptide of the seven-transmembrane domain TR (TR1-41) is a strong platelet agonist.

L25 ANSWER 11 OF 24 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1998:64925 CAPLUS
DOCUMENT NUMBER: 128:203357
TITLE: Identification of tropoelastin as a ligand for

the 65-kDa FK 506-binding protein, FKBP65, in the secretory pathway

AUTHOR(S): Davis, Elaine C.; Broekelmann, Thomas J.; Ozawa, Yuji; Mecham, Robert P.

CORPORATE SOURCE: Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO, 63110, USA

SOURCE: J. Cell Biol. (1998), 140(2), 295-303
CODEN: JCLBA3; ISSN: 0021-9525

PUBLISHER: Rockefeller University Press

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The folding and trafficking of tropoelastin is thought to be mediated by intracellular chaperones, although the identity and role of any tropoelastin chaperone remain to be detd. To identify proteins that are assocd. with tropoelastin intracellularly, **bifunctional** chem. crosslinkers were used to covalently stabilize interactions between tropoelastin and assocd. proteins in the secretory pathway in intact fetal bovine auricular chondrocytes. Immunopptn. of tropoelastin from cell lysates after crosslinking and anal. by SDS-PAGE showed the presence of two proteins of .apprx.74 kDa (p74) and 78 kDa (p78) that coimmunopptd. with tropoelastin. Microsequencing of peptide fragments from a cyanogen bromide digest of p78 identified this protein as BiP and sequence anal. identified p74 as the **peptidyl-prolyl** cis-trans **isomerase**, FKPB65. The appearance of BiP and FKBP65 in the immunopptns. could be enhanced by the addn. of brefeldin A (BFA) and N-acetyl-Leu-Leu-norleucinal (ALLN) to the culture medium for the final 4 h of labeling. Tropoelastin accumulates in the fused ER/Golgi compartment in the presence of BFA if its degrdn. is inhibited by ALLN (Davis, E.C.; Mecham, R.P., 1996). The use of BFA and other secretion-disrupting agents suggests that the assocn. of tropoelastin with FKBP65 occurs in the ER. Results from this study provide the first identification of a ligand for an FKBP in the secretory pathway and suggest that the prolyl cis-trans isomerase activity of FKBP65 may be important for the proper folding of the proline-rich tropoelastin mol. before secretion.

L25 ANSWER 12 OF 24 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1997:494416 CAPLUS

DOCUMENT NUMBER: 127:202629

TITLE: Nascent membrane and presecretory proteins synthesized in Escherichia coli associate with signal recognition particle and trigger factor

AUTHOR(S): Valent, Quido A.; De Gier, Jan-Willem L.; Von Heijne, Gunnar; Kendall, Debra A.; Ten Hagen-Jongman, Corinne M.; Oudega, Bauke; Luirink, Joen

CORPORATE SOURCE: Department of Microbiology, Biocentrum

Amsterdam, Amsterdam, 1081 HV, Neth.
SOURCE: Mol. Microbiol. (1997), 25(1), 53-64
CODEN: MOMIEE; ISSN: 0950-382X
PUBLISHER: Blackwell
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The E. coli signal recognition particle (SRP) and trigger factor are cytoplasmic factors that interact with short nascent polypeptides of presecretory and membrane proteins produced in a heterologous in vitro translation system. In this study, an E. coli in vitro translation system was used in combination with **bifunctional** crosslinking reagents to investigate these interactions in more detail in a homologous environment. Using this approach, the direct interaction of SRP with nascent polypeptides that expose particularly hydrophobic targeting signals is demonstrated, suggesting that inner membrane proteins are the primary physiol. substrate of the E. coli SRP. Evidence is presented that the overprodn. of proteins that expose hydrophobic polypeptide stretches, titrates SRP. In addn., trigger factor is efficiently cross-linked to nascent polypeptides of different length and nature, some as short as 57 amino acid residues, indicating that it is positioned near the nascent chain exit site on the E. coli ribosome.

IT **95076-93-0**
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(trigger factor; nascent membrane and presecretory proteins synthesized in Escherichia coli assoc. with signal recognition particle and trigger factor)

L25 ANSWER 13 OF 24 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1996:438429 CAPLUS
DOCUMENT NUMBER: 125:131894
TITLE: Catalytic lectin (leczyme) from bullfrog (Rana catesbeiana) eggs: Mechanism of tumoricidal activity
AUTHOR(S): Nitta, Kazuo; Ozaki, Kouichi; Tsukamoto, Yoshimasa; Hosono, Masahiro; Ogawa-Konno, Yukiko; Kawauchi, Hiroaki; Takayanagi, Yoshio; Tsuiki, Shigeru; Hakomori, Sen-Itiroh
CORPORATE SOURCE: Tohoku College Pharmaceutical Sciences, Cancer Research Institute, Sendai, 981, Japan
SOURCE: Int. J. Oncol. (1996), 9(1), 19-23
CODEN: IJONES; ISSN: 1019-6439
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Catalytic lectins (leczymes) of frog eggs are sialic acid-binding lectins that have intrinsic RNase activity. They inhibit tumor cell proliferation in vitro and in vivo, although their cytotoxic mechanism remains unclear. RNase A has no tumoricidal activity. It

is hypothesized that leczymes bind to cell surface sialoglycoconjugate receptors, enter the cell, and subsequently degrade RNA. In order to investigate the cytotoxic mechanism of cSBL, a leczyme from Rana catesbeiana eggs, we established cSBL-resistant clone RC-150 from mouse leukemia P388 cells. CSBL-treated P388 cells showed extensive RNA degrdn. over the course of 1 h, whereas cSBL-treated RC-150 cells showed no RNA degrdn. even over the course of 24 h. Treatment of P388 cells with cSBL led to decreased concn. of intracellular Ca2+, decreased protein kinase A activity, and increased protein kinase G activity. Incubation with cSBL decreased glutathione levels and enhanced glutathione-S-transferase (GST) activity in P388 cells, but had no effect on RC-150 cells. We conclude that cSBL-specific degrdn. of RNA occurs in cSBL-sensitive tumor cells, that cSBL leads to alteration of signal transduction and an **intracellular protein** kinase cascade reaction, and that internalized cSBL is detoxified by GST or thioltransferase. Our findings support a **bifunctional** model in which a leczyme is both an adhesive protein (binding to sialoglycoconjugates) and an enzyme (displaying RNase activity).

L25 ANSWER 14 OF 24 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1996:364354 CAPLUS
DOCUMENT NUMBER: 125:80924
TITLE: **Bifunctional** protein crosslinking reagents improve labeling of cytoskeletal proteins for qualitative and quantitative fluorescence microscopy
AUTHOR(S): Safiejko-Mroczka, Barbara; Bell, Paul B., Jr.
CORPORATE SOURCE: Dep. Zoology, Univ. Oklahoma, Norman, OK, USA
SOURCE: J. Histochem. Cytochem. (1996), 44(6), 641-656
CODEN: JHCYAS; ISSN: 0022-1554
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Because permeabilization of the cell membrane is necessary to label **intracellular proteins** with most fluorescent probes, it is important to optimize the preservation and labeling of the proteins under study. We used qual. and quant. fluorescence microscopy to evaluate the effects of six different **bifunctional** protein crosslinking reagents and several extn. conditions on the labeling of filament actin with phalloidin and the immunolabeling of tubulin and gelsolin. The labeling of cytoskeletal and assocd. proteins can be significantly enhanced by the appropriate combination of **bifunctional** protein crosslinking reagent and extn. conditions. However, the conditions that give the most intense vary depending on the label used. The greatest intensity of labeling with either phalloidin or antibodies was obtained with the intermediate-length cross-liner DSP. The two-step procedure of crosslinking with DSP and extg. in Triton

X-100 in microtubule with phalloidin. Maximal labeling of gelsolin and tubulin with antibodies is obtained by extn. DSP cross-linked cells with Triton in Hank's saline contg. DSP. Therefore, DSP reproducibly improves preservation of both sol. and filamentous proteins for quant. and qual. studies by fluorescence microscopy.

L25 ANSWER 15 OF 24 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1994:426409 CAPLUS
DOCUMENT NUMBER: 121:26409
TITLE: Synthetic peptide **ligands** of the antigen binding receptor induce programmed cell death in a human B-cell lymphoma
AUTHOR(S): Renschler, Markus F.; Bhatt, Ramesh R.; Dower, William J.; Levy, Ronald
CORPORATE SOURCE: Div. Oncol., Stanford Univ., Stanford, CA, 94305-5306, USA
SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1994), 91(9), 3623-7
CODEN: PNASA6; ISSN: 0027-8424
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Peptide **ligands** for the antigen binding site of the surface Ig receptor of a human B-cell lymphoma cell line were identified with the use of filamentous phage libraries displaying random 8- and 12-amino acid peptides. Corresponding synthetic peptides bound specifically to the antigen binding site of this Ig receptor and blocked the binding of an anti-idiotype antibody. The **ligands**, when **conjugated** to form dimers or tetramers, induced cell death by apoptosis in vitro with an IC50 between 40 and 200 nM. This effect was assocd. with specific stimulation of **intracellular protein** tyrosine phosphorylation.

L25 ANSWER 16 OF 24 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1993:616859 CAPLUS
DOCUMENT NUMBER: 119:216859
TITLE: Cytotoxicity of folate-Pseudomonas exotoxin **conjugates** toward tumor cells. Contribution of translocation domain
AUTHOR(S): Leamon, Christopher P.; Pastan, Ira; Low, Philip S.
CORPORATE SOURCE: Dep. Chem., Purdue Univ., West Lafayette, IN, 47907-1393, USA
SOURCE: J. Biol. Chem. (1993), 268(33), 24847-54
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Folate-protein **conjugates** can be nondestructively delivered into a cell's cytoplasm via folate receptor-mediated

endocytosis if (i) the target cells express a folate-binding protein, and (ii) if the folate is **linked** to its attached protein at a site that does not interfere with receptor recognition. Because such **conjugates** have been obsd. to remain in endosomal compartments for extended periods following cellular uptake, the authors decided to evaluate whether release into the cytoplasm might be expedited by inclusion of a translocation domain in the folate-protein construct. To test this possibility, momordin-folate and truncated Pseudomonas exotoxin-folate **c njugates** (LysPE38 and CysPE35), i.e. protein synthesis **inhibitors** either lacking or contg. the desired translocation domain, resp., were examd. for their abilities to **block protein** synthesis in a variety of cell types. The translocation competent LysPE38-folate construct was found to kill cells six times more rapidly with 10-fold greater potency than the permeation-incompetent momordin-folate. Further, cells expressing low levels of folate receptors could only be exterminated by the translocation competent Pseudomonas exotoxin-folate **conjugates**. When the translocation capability of CysPE35-folate was inactivated by modification of Cys287, the construct also lost most of its cytotoxicity. These data suggest that autocatalysis of transport from an internal vesicular compartment into the cytoplasm can greatly augment the cytotoxicity of a protein toxin entering cells via the folate endocytosis pathway. Because the folate **ligand** can selectively target a protein **conjugate** to cancer cells in the presence of normal cells, such translocatable toxin-folate constructs warrant further study as a possible treatment for some malignancies.

L25 ANSWER 17 OF 24 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1993:534115 CAPLUS
DOCUMENT NUMBER: 119:134115
TITLE: ATPase-promoting dead end inhibitors of the cAMP-dependent protein kinase
AUTHOR(S): Mendelow, Marianne; Prorok, Mary; Salerno, Allen; Lawrence, David S.
CORPORATE SOURCE: Dep. Chem., State Univ. New York, Buffalo, NY, 14214, USA
SOURCE: J. Biol. Chem. (1993), 268(17), 12289-96
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The cAMP-dependent protein kinase is a **bifunctional** enzyme, catalyzing the phosphorylation of the serine and threonine residues in peptides and proteins (kinase activity) as well as the phosphorylation of water (ATPase activity). The authors have found that several peptides, which serve as inhibitors of the kinase reaction, will either maintain or enhance the ATPase reaction

catalyzed by the enzyme. Pos. charged dipeptides (e.g. Arg-Arg), as well as small guanidino contg. compds. (e.g. guanethidine) **block protein** kinase activity yet enhance ATPase activity up to 3.5-fold over that exhibited by the enzyme in the absence of these compds. In contrast, several nonphosphorylatable peptides, whose primary sequences are based on that of a known substrate (i.e. Leu-Arg-Arg-Ala-Ser-Leu-Gly), such as Leu-Arg-Arg-Ala-Ala-Leu-Gly, Leu-Arg-Arg-Ala-Phe-Leu-Gly, and Leu-Arg-Arg-Ala-Tyr-Leu-Gly, have little or no effect on the rate of the kinase-catalyzed hydrolysis of ATP. An exception to the latter observation is Leu-Arg-Arg-Ala-Cys-Leu-Gly, a cysteine-contg. peptide that promotes the protein kinase-catalyzed ATPase reaction by 2.2-fold. The authors have also found that peptides that possess relatively large amino acid side chain moieties immediately following the arginine dyad (i.e. such as Phe, Tyr, Cys, or Asn at Xaa in Leu-Arg-Arg-Xaa-Ala-Leu-Gly) sharply reduce the rate of enzyme-catalyzed ATP hydrolysis. This suggests that in the presence of peptides contg. an -Arg-Arg-Ala- sequence, the enzyme-bound .gamma.-phosphate of ATP is relatively accessible to water. In contrast, when the the latter alanine moiety is replaced by a larger residue, access by water to ATP appears to be hindered. These results indicate that certain structural features assocd. with the substrate or substrate analog have a profound influence on the manner by which these species interact with the protein kinase. Furthermore, the work described herein demonstrates that it is possible to block the physiol. important kinase reaction and simultaneously promote the energetically wasteful ATPase reaction.

L25 ANSWER 18 OF 24 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1993:146017 CAPLUS
DOCUMENT NUMBER: 118:146017
TITLE: Cross-**linking** of CD14 molecules on monocytes results in a CD11/CD18- and ICAM-1-dependent adherence to cytokine-stimulated human endothelial cells
AUTHOR(S): Beekhuizen, Henry; Blokland, Irene; Van Furth, Ralph
CORPORATE SOURCE: Dep. Infect. Dis., Univ. Hosp., Leiden, 2300, Neth.
SOURCE: J. Immunol. (1993), 150(3), 950-9
CODEN: JOIMA3; ISSN: 0022-1767
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The myeloid differentiation protein CD14 that is expressed on the surface of mature monocytes contributes to the adherence of monocytes to cytokine-stimulated monolayers of human macrovascular endothelial cells (EC). It has also been obsd. that the initial adherence of monocytes to cultured cytokine-stimulated EC eventually results in an ICAM-1- and LFA-1 (CD11a/CD18)-dependent adherence,

which coincides with stretching and lateral migration of the monocytes over the surface of EC. Recently, it was reported that CD14 mediates monocyte activation and can induce a change in the avidity of CD11a/CD18 for its **ligand** ICAM-1. The aim of the present study was to investigate whether activation of monocytes by CD14 elicits a CD11/CD18-dependent adhesion of monocytes to ICAM-1 on rIL-1.alpha.-stimulated EC. Incubation of monocytes with murine anti-CD14 mAb alone did not mobilize intracellular calcium but the subsequent addn. of F(ab')2 anti-mouse Ig, which caused crosslinking of CD14 on the surface of monocytes, induced a transient rise in cytosolic free calcium concn. and enhanced the percentage monocytes that adhered to monolayers of macrovascular venous EC stimulated with rIL-1.alpha. for 24 h, but not to nonstimulated EC. The elevated adhesion was decreased when monocytes were preincubated with staurosporine, an **inhibitor** of **intracellular protein** kinase activity and was markedly inhibited by mAb against the common .beta.2-subunit (CD18) of the CD11/CD18 mols. on monocytes and by mAb against ICAM-1 on 24-h rIL-1.alpha.-stimulated venous EC. Thus, the binding of monocytes via CD14 to rIL-1.alpha.-stimulated EC generates an intracellular response in monocytes and triggers an adhesion mechanism that allows CD11/CD18 mols. on monocytes to bind to ICAM-1 on EC.

L25 ANSWER 19 OF 24 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1988:627946 CAPLUS
DOCUMENT NUMBER: 109:227946
TITLE: Abnormal proteins as the trigger for the induction of stress responses: heat, diamide, and sodium arsenite
AUTHOR(S): Lee, Kong Joo; Hahn, George M.
CORPORATE SOURCE: Dep. Radiat. Oncol., Stanford Univ., Stanford, CA, 94305-5468, USA
SOURCE: J. Cell. Physiol. (1988), 136(3), 411-20
CODEN: JCLLAX; ISSN: 0021-9541
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Thermotolerance and synthesis of heat shock proteins are induced in cells in response to a variety of environmental stresses. The suggestion that modifications of **intracellular proteins** may be the triggering event that induces heat-shock protein synthesis and thermotolerance was examd. by modifying cellular proteins with diamide, a sulfhydryl oxidizing agent, and with dithio-bis (succinimidyl propionate), an agent that cross-links **bifunctional** amino groups. Both of these agents induced heat-shock proteins and thermotolerance in CHO (HA-1) cells. Furthermore, cross-resistance and self-tolerance with three seemingly unrelated stimuli (diamide, heat, and sodium arsenite) were obsd. Apparently the induction of protective responses to

these stimuli is mediated by a common mechanism. The results support the hypothesis that prodn. of abnormal proteins by various stresses induces the stress responses as well as tolerance.

L25 ANSWER 20 OF 24 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1988:435772 CAPLUS
DOCUMENT NUMBER: 109:35772
TITLE: Crosslinking of .alpha.- and .gamma.-thrombin to distinct binding sites on human platelets
AUTHOR(S): Jandrot-Perrus, Martine; Didry, Dominique; Guillin, Marie Claude; Nurden, Alan T.
CORPORATE SOURCE: Lab. Rech. Hemostase Thrombose, Fac. Xavier Bichat, Paris, Fr.
SOURCE: Eur. J. Biochem. (1988), 174(2), 359-67
CODEN: EJBCAI; ISSN: 0014-2956
DOCUMENT TYPE: Journal
LANGUAGE: English

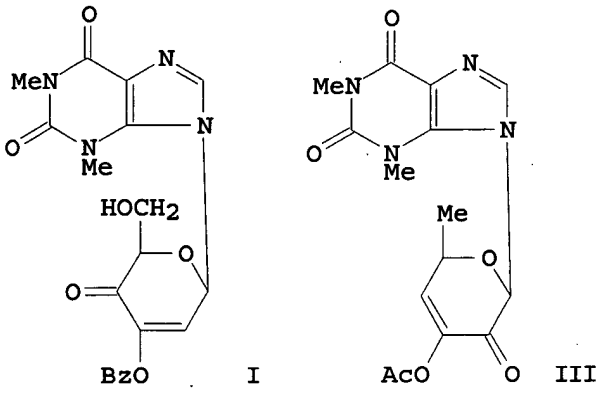
AB The interaction of thrombin with proteins at the platelet surface was assessed by chem. crosslinking with the membrane-impermeable reagents bis(sulfosuccinimidyl)suberate and dithiobis(sulfosuccinimidyl propionate) under conditions which induced no modification of **intracellular proteins** and minimal crosslinking of membrane glycoproteins. The proteins covalently linked to 125I-labeled .alpha.- and .gamma.-thrombin were analyzed by SDS/PAGE and crossed immunoelectrophoresis. 125I-labeled .alpha.-thrombin was detected in high-mol.-mass complexes (1) at the top of a 3% acrylamide stacking gel and (2) with a mol. wt. of .apprxeq.400,000. In addn., 2 complexes of 240 kilodaltons (kDa) and 78 kDa were characterized. Hirudin prevented the formation of each of these complexes. The 78-kDa complex occurred spontaneously in the absence of **bifunctional** reagents, was only obsd. with active .alpha.-thrombin, and was not dissocd. by hirudin. Such characteristics are similar to those of a serpin serine-protease complex. The 240-kDa complex was formed with 0.8-100 nM .alpha.-thrombin, was obsd. after a short incubation time (30 s), and occurred with N-tosyl-L-lysylchloromethane-inactivated .alpha.-thrombin. After anal. of Triton X 100-sol. exts. of crosslinked platelets by crossed immunoelectrophoresis against a rabbit antiserum to platelets, 2 principal ppts. contained 125I-labeled .alpha.-thrombin. These were a ppt. contg. glycoprotein (GP) IIb-IIIa complexes and a ppt. in the position of GPIb. Indirect immunopptn. of GPIb, by using a murine monoclonal antibody, confirmed it to be the major platelet component in the 240-kDa complex. Significantly, 125I-labeled .gamma.-thrombin, which activates platelets with a prolonged lag phase, failed to bind to GPIb, and complexes in the 240-kDa and 78-kDa mol. mass range were not obsd. Hence, several binding sites for .alpha.-thrombin are present at the platelet surface, and GPIb is one of them. The studies with .gamma.-thrombin suggest that binding to GPIb is not

obligatory for platelet activation although it could be involved in an initial step of the platelet response.

L25 ANSWER 21 OF 24 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1987:492681 CAPLUS
DOCUMENT NUMBER: 107:92681
TITLE: Ribonucleotide reductase of Escherichia coli. Cross-linking agents as probes of quaternary and quinary structure
AUTHOR(S): Mathews, Christopher K.; Sjoeberg, Britt Marie; Reichard, Peter
CORPORATE SOURCE: Dep. Biochem. Biophys., Oregon State Univ., Corvallis, OR, 97331, USA
SOURCE: Eur. J. Biochem. (1987), 166(2), 279-85
CODEN: EJBCAI; ISSN: 0014-2956
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The quaternary structure of ribonucleotide reductase of E. coli was investigated by use of purified B1 and B2 proteins and **bifunctional** crosslinking agents. The holoenzyme is an .alpha.2.beta.2 tetramer consisting of 2 dimeric proteins: B1 (.alpha.2) and B2 (.beta.2). The crosslinking data support a model in which both of the .beta. subunits interact closely with only 1 of the 2 .alpha. subunits. Some of the interactions involving B2 were localized to the C terminus of the protein by use of truncated B2 protein (.beta.'.beta.'), a proteolytic cleavage product of B2 in which the 30 C-terminal residues are missing from each of the .beta. subunits. Other interactions were indicated by the ability of glutaredoxin, but not thioredoxin, to inhibit some of the crosslinking reactions. Ribonucleotide reductase interaction with other proteins inside the cell was examd. by adding crosslinkers directly to suspensions of whole bacteria. Proteins of these crosslinked bacteria were resolved electrophoretically and probed with a monoclonal antibody to the B1 protein. High-mol.-mass products were detected, supporting the utility of this method for identifying intracellular interactions among enzymes of DNA precursor biosynthesis.

L25 ANSWER 22 OF 24 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1984:29301 CAPLUS
DOCUMENT NUMBER: 100:29301
TITLE: Interactions of cytostatic unsaturated ketonucleosides with sulfhydryl containing cell constituents
AUTHOR(S): Halmos, T.; Cardon, A.; Antonakis, K.
CORPORATE SOURCE: Inst. Rech. Sci. Cancer, Villejuif, 94802, Fr.
SOURCE: Chem.-Biol. Interact. (1983), 46(1), 11-29
CODEN: CBINA8; ISSN: 0009-2797
DOCUMENT TYPE: Journal

LANGUAGE: English
GI



I III

AB The cytostatic unsatd. ketonucleosides I [88332-05-2] and its 5-deoxy deriv. (II) [88332-06-3] as well as III [85155-73-3] and its 3-bromo-.beta.-deriv. (IV) [88375-92-2] are highly reactive SH-blocking agents. Kinetics of their reactions with GSH [70-18-8] were measured and their reactivity was compared to that of N-ethylmaleimide (NEM), acrylonitrile, and chloroacetamide. Their reaction products with N-acetyl-L-cysteine (AcCys) were prepd. and characterized by chem. anal. and NMR spectroscopy. I, II, and III gave Michael type 1:1 addn. products. IV reacted with AcCys by a 3-step mechanism. In the reaction with GSH, IV behaved like a **bifunctional** SH-alkylating agent. I, II, III, and IV also reacted with protein thiols, as shown by their ability to inhibit lactate dehydrogenase [9001-60-9]. Unsatd. ketonucleosides had diverse effects on L1210 leukemia cells. While the most potent cytostatics, I and III, reduced considerably the membrane surface SH level, they were without effect on sol. **intracellular protein** thiols. In contrast, nucleosides II and IV, less active than the former, only slightly affected the membrane surface SH groups and considerably depleted the intracellular sol. protein thiols. Only slight differences were found between the reactions of the 4 nucleosides with nonprotein SH. The correlation found between in vivo biol. activity and cell membrane impairment suggests that selective alkylation of certain key membrane thiols by unsatd. ketonucleosides might be an important event in their biol. effect.

L25 ANSWER 23 OF 24 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1981:44654 CAPLUS
DOCUMENT NUMBER: 94:44654
TITLE: Permeabilization of animal cells for kinetic studies of intracellular enzymes: In situ

Searcher : Shears 308-4994

behavior of the glycolytic enzymes of erythrocytes
AUTHOR(S): Aragon, Juan J.; Feliu, Juan E.; Frenkel, Rene A.; Sols, Alberto
CORPORATE SOURCE: Fac. Med., Univ. Auton., Madrid, Spain
SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1980), 77(11), 6324-8
CODEN: PNASA6; ISSN: 0027-8424
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Intracellular enzymes in erythrocytes can be made accessible for in situ kinetic studies by treating the cells with **bifunctional** reagents to crosslink proteins, thus creating a network that allows subsequent permeabilization by delipidation without escape of **intracellular proteins**. Di-Me suberimidate, di-Me 3,3'-dithiobispropionimidate, and toluene-2,4-diisocyanate were used successfully as crosslinking reagents, and digitonin was used for the delipidation. In a systematic study of the in situ behavior of the 11 glycolytic enzymes of rat erythrocytes, it was obsd. that Km and Vmax values for the majority of the enzymes are essentially the same in situ as in vitro. Lactate dehydrogenase (EC 1.1.1.27) is inhibited by excess of pyruvate as much in situ as in vitro. Hexokinase (EC 2.7.1.1) was allosterically inhibited by glucose 6-phosphate nearly as much as in situ as in vitro but was not affected by 2,3-biphosphoglycerate. The allosteric properties of 6-phosphofructokinase (EC 2.7.1.11), glyceraldehyde-phosphate dehydrogenase (EC 1.2.1.12), and pyruvate kinase (EC 2.7.1.40) in situ were qual. similar to those obsd. in vitro, but some important quant. differences were noticed. Particularly striking was the much greater activity of phosphofructokinase in situ compared to that in vitro at physiol. concns. of effector metabolites.

L25 ANSWER 24 OF 24 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1976:555535 CAPLUS
DOCUMENT NUMBER: 85:155535
TITLE: **Intracellular protein** breakdown. VII. Cathepsin L and H: two new proteinases from rat liver lysosomes
AUTHOR(S): Kirschke, H.; Langner, J.; Wiederanders, B.; Ansorge, S.; Bohley, P.; Broghammer, U.
CORPORATE SOURCE: Physiol.-Chem. Inst., Martin-Luther-Univ., Halle/Saale, E. Ger.
SOURCE: Acta Biol. Med. Ger. (1976), 35(3-4), 285-99
CODEN: ABMGAJ
DOCUMENT TYPE: Journal
LANGUAGE: German
AB Some properties (mol. wt., pI, temp. stability, action of selected inhibitors, substrate specificity, and pH-activity dependence) of 2 not previously reported cathepsins from rat liver lysosomes were

compared with the properties of the known cathepsin B1. Cathepsin L was a sulfhydryl proteinase, had a mol. wt. of 23,000-24,000 and a pI of 5.8-6.1. By disc electrophoresis and isoelec. focusing several protein bands which all had enzymic activity were obsd. Leupeptin was a strong inhibitor. The pH-optimum for digestion of proteins was close to 5,0. Cathepsin L did not hydrolyze esters and cleaved synthetic low- mol.-wt. substrates only to a small degree. Cathepsin L stored in presence of glutathione and EDTA in liq. nitrogen kept its activity for several months. Cathepsin H was an aminopeptidase as well as an endopeptidase. An enzyme with these **bifunctional** properties had been detected up to now only in Escherichia coli but not in animal cells. Cathepsin H was a sulfhydryl enzyme with a mol. wt. of 28,000 and a pI1 of 7.,1. Leucylchloromethane and SH-blocking agents were strong inhibitors. Leupeptin showed only a weak inhibitory effect on this enzyme compared to its action on cathepsins L und B1. The pH-optimum for hydrolysis of all substrates was 6.0. Cathepsin H cleaved proteins, amino acid derivs., and selected N-protected amino acid derivs. Cathepsin H was quite temp. stable compared to cathepsins L and B1.

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, JAPIO' ENTERED AT 09:36:36 ON 16 MAR 2001)

L26 77 S L25
L27 29 DUP REM L26 (48 DUPLICATES REMOVED)

L27 ANSWER 1 OF 29 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2000-452123 [39] WPIDS
CROSS REFERENCE: 2000-350827 [30]
DOC. NO. NON-CPI: N2000-336634
DOC. NO. CPI: C2000-137757
TITLE: Identifying **ligands** for the farnesoid X receptor, useful as potential agents for treating e.g. atherosclerosis and obesity, comprises measuring receptor binding with co-activator peptide.
DERWENT CLASS: B03 B04 S03
INVENTOR(S): BLANCHARD, S G; KLIEWER, S A; LEHMANN, J; PARKS, D J; STIMMEL, J B; WILLSON, T M
PATENT ASSIGNEE(S): (GLAX) GLAXO GROUP LTD
COUNTRY COUNT: 90
PATENT INFORMATION:

| PATENT NO | KIND | DATE | WEEK | LA | PG |
|---|---|---|---|---|---|
| WO 2000037077 | A1 | 20000629 | (200039)* | EN | 61 |

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ

LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU
SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
AU 2000023891 A 20000712 (200048)

APPLICATION DETAILS:

| PATENT NO | KIND | APPLICATION | DATE |
|---|---|---|---|
| WO 2000037077 A1 | | WO 1999-US30947 | 19991222 |
| AU 2000023891 A | | AU 2000-23891 | 19991222 |

FILING DETAILS:

| PATENT NO | KIND | PATENT NO |
|---|---|---|
| AU 2000023891 A | Based on | WO 200037077 |

PRIORITY APPLN. INFO: US 1998-135097 19981223
AN 2000-452123 [39] WPIDS
CR 2000-350827 [30]
AB WO 200037077 A UPAB: 20001001
NOVELTY - Rapid detection of **ligands** for the farnesoid X receptor (FXR) comprises applying test compound (I) to (i) a FXR-**ligand** binding domain (LBD), associated with a first marker (M1) and (ii) a nuclear receptor co-activating peptide (II), associated with a second marker (M2). Interaction between M1 and M2 is measured to determine if (I) modifies the binding between (II) and the LBD.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(a) similar method as above in which (I) is treated with (i) a nuclear receptor LBD labeled with M1 and (ii) a heterodimeric partner for (i), labeled with M2, and measuring any interaction between M1 and M2 to determine if (I) modulates heterodimerization;

(b) method for identifying compounds for treating disorders modulated by FXR by identifying compounds that interact directly with FXR;

(c) compounds (L) identified by any of the new methods;

(d) compounds of formula (La), optionally labeled, that bind FXR;

X1 = CH or N;

X2 = O or NH;

R, R1 = H, lower alkyl, halo or trifluoromethyl;

R2 = lower alkyl;

R3, R4 = H, lower alkyl, halo, trifluoromethyl, hydroxy, alkoxy or polyhaloalkoxy;

(e) regulating I-BABP (intestinal bile acid binding protein) expression in a mammal by activating or inhibiting FXR;

(f) regulating the bile acid transport system in a mammal by

activating FXR with a binding **ligand**;

(g) method for treating, in mammals, diseases affected by levels of cholesterol, triglycerides or bile acid by administering a FXR **ligand**;

(h) method for blocking fatty acid adsorption in the intestine of a mammal by administering an FXR agonist;

(j) method for **blocking protein** and carbohydrate digestion in the intestine of a mammal by administering an FXR agonist;

(k) method for blocking de novo cholesterol synthesis in the liver of a mammal by administering an FXR antagonist;

(l) method for blocking induction of SHP-1 expression in a mammal by administering an FXR antagonist;

(m) method for blocking SHP-1 mediated repression of CYP7A in a mammal by administering an SHP-1 antagonist;

(n) use of RXR (retinoid X receptor)-specific **ligands** for treating disorders modulated by FXR; and

(o) method for modulating an FXR-regulated gene by administering an FXR **ligand**.

ACTIVITY - Antilipemic; antiarteriosclerotic; litholytic; hepatotropic; cardiant; anorectic.

MECHANISM OF ACTION - Modulation of FXR which is involved in regulation of many genes involved in bile acid, lipid and cholesterol homeostasis, fatty acid absorption and digestion of proteins and carbohydrates.

USE - The **ligands** are used for treating FXR-related diseases, particularly those related to levels of bile acids, triglycerides and cholesterol, e.g. atherosclerosis, gall stones, lipid disorders, cardiovascular diseases and obesity.

ADVANTAGE - **Ligands** can be identified rapidly and simply.

Dwg.0/5

L27 ANSWER 2 OF 29 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2000-339649 [29] WPIDS
DOC. NO. CPI: C2000-103102
TITLE: Generating a peptide with a selected biological activity useful for identifying endothelial **inhibitors** and peptides with anti-angiogenic activity by combining peptide display libraries in a display and a secretion mode.
DERWENT CLASS: B04 D16
INVENTOR(S): GYURIS, J; MORRIS, A J
PATENT ASSIGNEE(S): (MITO-N) MITOTIX INC
COUNTRY COUNT: 88
PATENT INFORMATION:

| PATENT NO | KIND | DATE | WEEK | LA | PG |
|---|---|---|---|---|---|

---

WO 2000023465 A2 20000427 (200029)* EN 86
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC
MW NL OA PT SD SE SL SZ TZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES
FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK
LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE
SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
AU 2000013164 A 20000508 (200037)

APPLICATION DETAILS:

| PATENT NO | KIND | APPLICATION | DATE |
|---|---|---|---|
| WO 2000023465 A2 | | WO 1999-US24276 | 19991019 |
| AU 2000013164 A | | AU 2000-13164 | 19991019 |

FILING DETAILS:

| PATENT NO | KIND | | PATENT NO |
|---|---|---|---|
| AU 2000013164 A | Based on | | WO 200023465 |

PRIORITY APPLN. INFO: US 1998-174943 19981019
AN 2000-339649 [29] WPIDS
AB WO 200023465 A UPAB: 20000617
NOVELTY - Generating a peptide having a selected biological activity comprises displaying the peptides on the outer surface of a genetic display package to create a peptide display library, and using affinity selection to enrich the population display packages for those containing peptides which have desired specificity to the target cell.

DETAILED DESCRIPTION - Generating a peptide with selected biological activity comprises:

(a) providing a peptide display library of test peptides expressed on the surface of display packages;

(b) in the display mode, isolating from the library a sub-population of display packages enriched for test peptides having a desired cell binding specificity or affinity for a cell or its component;

(c) in a secretion mode, simultaneously expressing the enriched test peptide sub-population, so the test peptides are secreted and free of the display packages; and

(d) testing the ability of the secreted test peptides to regulate a biological process in a target cell.

INDEPENDENT CLAIMS are also included for the following:

(1) a peptide display library enriched for test peptides having a desired binding specificity or affinity for a cell or its component, which regulate a biological process in target cell;

(2) a vector comprising a chimeric gene for a chimeric protein, the chimeric gene comprises:

(i) a coding sequence for a test peptide;

(ii) a coding sequence for a surface protein of a display package; and

(iii) RNA splice sites flanking the coding sequence for the surface protein, where in a display mode, the chimeric gene is expressed as a fusion protein including the test peptide and the surface protein such that the test peptide can be displayed on the surface of a population of display packages, whereas in the secretion mode, the test peptide is expressed without the surface protein as a result of the coding sequence for the surface protein being removed by RNA splicing;

(3) a vector library collectively encoding a variegated population of test peptides, where each vector comprises (2);

(4) a cell composition comprising a population of cells containing (3);

(5) a method for generating a peptide with a selected antimicrobial activity comprising:

(a) providing a recombinant host cell population which expresses a soluble peptide library comprising a variegated population of test peptides;

(b) culturing the host cells with a target microorganism so the peptide library is secreted and diffuses to the target microorganism; and

(c) selecting host cells expressing test peptides that inhibit growth of the target microorganism;

(6) methods for modulating an angiogenic process, and preventing or treating infection of an animal by a microorganism by administering a composition containing one or more test peptides or peptidomimetics; and

(7) the constructs pAM6 M13/COS, pAM7 and pAM9 M13/COS, and pAM8 M13/COS.

USE - The method may be used in the selection of peptides having effects on cell proliferation, differentiation, death and migration, as well as in the identification of peptides which have anti-proliferative activity with respect to one or more types of cells, peptides with (anti-)angiogenic activity, anti-infective peptides (e.g. which are active as anti-fungal or anti-bacterial), receptor protein effectors, and **ligands** for orphan receptors for which no **ligand** is known. Moreover, the method may be used to test functional **ligand**-receptor or **ligand**-ion channel interactions for cell surface-localized receptors and channels.

ADVANTAGE - The new method allows re-synthesis and re-screening of molecules with a desired binding activity, and can lead to isolation and identification of peptides with specific activities from a large pool of molecules. The use of the method reduces the loss of peptide sequences from the sub-library by eliminating

sub-cloning steps.
Dwg.0/16

L27 ANSWER 3 OF 29 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 2000:943984 SCISEARCH
THE GENUINE ARTICLE: 381AH
TITLE: Structural and functional characterization of the transforming growth factor-beta-induced Smad3/c-Jun transcriptional cooperativity
AUTHOR: Qing J; Zhang Y; Derynck R (Reprint)
CORPORATE SOURCE: UNIV CALIF SAN FRANCISCO, DEPT GROWTH & DEV, SAN FRANCISCO, CA 94143 (Reprint); UNIV CALIF SAN FRANCISCO, DEPT GROWTH & DEV, SAN FRANCISCO, CA 94143; UNIV CALIF SAN FRANCISCO, DEPT ANAT, CELL BIOL PROGRAM, SAN FRANCISCO, CA 94143; UNIV CALIF SAN FRANCISCO, DEPT ANAT, PROGRAM DEV BIOL, SAN FRANCISCO, CA 94143
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (8 DEC 2000) Vol. 275, No. 49, pp. 38802-38812.
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814.
ISSN: 0021-9258.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 58

*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Smads are **intracellular proteins** that act as central effecters for transforming growth factor-beta (TGF-beta) and related proteins from the activated receptor into the nucleus, where they regulate **Ligand**-induced gene expression. AP-1 binding sites have been functionally **linked** to the transcriptional activation of various genes in response to TGF-beta. Accordingly, we have previously shown that the heteromeric complex of Smad3 and Smad4 synergizes with c-Jun/c-Fos at the AP-1 binding site of the collagenase I promoter to induce transcriptional activation in response to TGF-beta. Using the collagenase I promoter as model system, we have now investigated the role of the c-Jun and Smad3 interactions with the promoter DNA and have further characterized the physical basis of the c-Jun/Smad3 interaction in the transcriptional response. Mutational analyses of the c-Jun protein and the AP-1 binding site in the promoter revealed that the interaction of c-Jun with DNA is necessary for transcriptional activation by TGF-beta and Smads. Similar analyses of Smad3 and the Smad binding sites revealed that binding of Smad3 to DNA is also required, but that its DNA sequence-specific recognition is not essential. We also found that the basic leucine zipper domain of c-Jun and a short sequence close to the N terminus of Smad3 mediate

their physical interaction, and that these regions are critical for their DNA-binding function. Our studies provide a basis for understanding the functional cooperativity of Smads with the diversity of transcription factors, which underlies the Smad-induced transcriptional activation in response to TGF-beta and related factors.

L27 ANSWER 4 OF 29 MEDLINE
ACCESSION NUMBER: 2000281319 MEDLINE
DOCUMENT NUMBER: 20281319
TITLE: Monomeric midkine induces tumor cell proliferation in the absence of cell-surface proteoglycan binding.
AUTHOR: Qiu L; Escalante C R; Aggarwal A K; Wilson P D; Burrow C R
CORPORATE SOURCE: Department of Medicine, The Mount Sinai School of Medicine, New York, NY 10029-6574, USA.
CONTRACT NUMBER: 1F32 DK09776 (NIDDK)
SOURCE: BIOCHEMISTRY, (2000 May 23) 39 (20) 5977-87. Journal code: A0G. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200008
ENTRY WEEK: 20000804

AB Midkine (MK), a retinoic acid-inducible heparin-binding protein, is a mitogen which initiates a cascade of **intracellular protein** tyrosine phosphorylation mediated by the JAK/STAT pathway after binding to its high affinity p200(+)/MKR cell surface receptor in the G401 cell line [Ratovitski, E. A. (1998) J. Biol. Chem. 273, 3654-3660]. In this study, we determined the biophysical characteristics of purified recombinant murine MK and analyzed the requirements for **ligand** multimerization and cell surface proteoglycan binding for the G401 cell mitogenic activity of MK. Our studies indicate that the secreted form of MK (M = 13 kDa) exists in solution as an asymmetric monomer with a frictional coefficient of 1. 48 and a Stokes radius of 23.7 A. By constructing bead models of MK using the program AtoB and the program HYDRO to predict the hydrodynamic properties of each model, our data suggest that MK has a dumb-bell shape in solution composed of independent N- and C-terminal domains separated by an extended **linker**. This asymmetric MK monomer is a biologically active **ligand** with mitogenic activity on G401 cells in vitro. Neither heparin-induced formation of noncovalent MK multimers nor tissue transglutaminase II covalent multimerization of MK enhanced MK mitogenic activity in this system. Since neither heparin competition nor cell treatment with chondroitinase ABC or heparinase III abolished the mitogenic effects of MK on G401 cells, cell-surface proteoglycan binding by MK does not appear to be a requirement for its observed mitogenic

effects. These results provide strong evidence that the MK-specific p200(+)/MKR has distinctive biochemical properties which distinguish it from the receptor tyrosine phosphatase cell-surface proteoglycan PTPzeta/RPTPbeta and support the hypothesis that the diverse biological effects of MK are mediated by multiple cell-specific signal transduction receptors.

L27 ANSWER 5 OF 29 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 1
ACCESSION NUMBER: 2001:21258 BIOSIS
DOCUMENT NUMBER: PREV200100021258
TITLE: Proteasome **inhibitor**-induced apoptosis of glioma cells involves the processing of multiple caspases and cytochrome c release.
AUTHOR(S): Wagenknecht, Bettina; Hermisson, Mirjam; Groscurth, Peter; Liston, Peter; Krammer, Peter H.; Weller, Michael (1)
CORPORATE SOURCE: (1) Laboratory of Molecular Neuro-Oncology, Department of Neurology, Medical School, University of Tuebingen, Hoppe-Seyler-Strasse 3, 72076, Tuebingen: michael.weller@uni-tuebingen.de Germany
SOURCE: Journal of Neurochemistry, (December, 2000) Vol. 75, No. 6, pp. 2288-2297. print.
ISSN: 0022-3042.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The proteasome is a multiprotein complex that is involved in the **intracellular protein** degradation in eukaryotes. Here, we show that human malignant glioma cells are susceptible to apoptotic cell death induced by the proteasome **inhibitors**, MG132 and lactacystin. The execution of the apoptotic death program involves the processing of caspases 2, 3, 7, 8, and 9. Apoptosis is inhibited by ectopic expression of X-**linked** **inhibitor** of apoptosis (XIAP) and by coexposure to the broad-spectrum caspase **inhibitor**, benzoyl-VAD-fluoromethyl ketone (zVAD-fmk), but not by the preferential caspase 8 **inhibitor**, crm-A. It is interesting that specific morphological alterations induced by proteasome inhibition, such as dilated rough endoplasmic reticulum and the formation of cytoplasmic vacuoles and dense mitochondrial deposits, are unaffected by zVAD-fmk. Apoptosis is also inhibited by ectopic expression of Bcl-2 or by an **inhibitor** of protein synthesis, cycloheximide. Further, cytochrome c release and disruption of mitochondrial membrane potential are prominent features of apoptosis triggered by proteasome inhibition. Bcl-2 is a stronger **inhibitor** of cytochrome c release than zVAD-fmk. XIAP and crm-A fail to modulate cytochrome c release. These data place cytochrome c release downstream of Bcl-2 activity but upstream of XIAP- and crm-A-sensitive caspases. The partial inhibition of cytochrome c

release by zVAD-fmk indicates a positive feedback loop that may involve cytochrome c release and zVAD-fmk-sensitive caspases. Finally, death **ligand**/receptor interactions, including the CD95/CD95 **ligand** system, do not mediate apoptosis induced by proteasome inhibition in human malignant glioma cells.

L27 ANSWER 6 OF 29 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 2000389292 MEDLINE
DOCUMENT NUMBER: 20272850
TITLE: Apoptosis induced by death receptors.
AUTHOR: Schneider P; Tschopp J
CORPORATE SOURCE: Institute of Biochemistry, University of Lausanne, Epalinges, Switzerland.. pascal.schneider@ib.unil.ch
SOURCE: PHARMACEUTICA ACTA HELVETIAE, (2000 Mar) 74 (2-3) 281-6. Ref: 26
Journal code: P0E. ISSN: 0031-6865.
PUB. COUNTRY: Switzerland
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
ENTRY MONTH: 200010
ENTRY WEEK: 20001002

AB Death receptors belong to the TNF receptor family and are characterised by an intracellular death domain that serves to recruit adapter proteins such as TRADD and FADD and cysteine proteases such as Caspase-8. Activation of Caspase-8 on the aggregated receptor leads to apoptosis. Triggering of death receptors is mediated through the binding of specific **ligands** of the TNF family, which are homotrimeric type-2 membrane proteins displaying three receptor binding sites. There are various means of modulating the activation of death receptors. The status of the **ligand** (membrane-bound vs. soluble) is critical in the activation of Fas and of TRAIL receptors. Cleavage of membrane-bound FasL to a soluble form (sFasL) does not affect its ability to bind to Fas but drastically decreases its cytotoxic activity. Conversely, cross-**linking** epitope-tagged sFasL with anti-tag antibodies to mimic membrane-bound **ligand** results in a 1000-fold increase in cytotoxicity. This suggests that more than three Fas molecules need to be aggregated to efficiently signal apoptosis. Death receptors can also be regulated by decoy receptors. The cytotoxic **ligand** TRAIL interacts with five receptors, only two of which (TRAIL-R1 and -R2) have a death domain. TRAIL-R3 is anchored to the membrane by a glycolipid and acts as a dominant negative **inhibitor** of TRAIL-mediated apoptosis when overexpressed on TRAIL-sensitive cells. **Intracellular proteins** interacting with the apoptotic pathway are potential modulators of death receptors. FLIP resembles Caspase-8 in structure but lacks protease activity. It interacts with both FADD

and Caspase-8 to inhibits the apoptotic signal of death receptors and, at the same time, can activate other signalling pathways such as that leading to NF-kappa B activation.

L27 ANSWER 7 OF 29 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2000-097072 [08] WPIDS
DOC. NO. CPI: C2000-028124
TITLE: **Bifunctional** molecule for incorporation into pharmaceutical compositions used in the treatment of cellular proliferative diseases, such as neoplastic, autoimmune and cardiovascular diseases.
DERWENT CLASS: B04 D16
INVENTOR(S): BRIESEWITZ, R; CRABTREE, G R; RAY, G T; VOGEL, K W; WANDLESS, T
PATENT ASSIGNEE(S): (STRD) UNIV LELAND STANFORD JUNIOR
COUNTRY COUNT: 86
PATENT INFORMATION:

| PATENT NO | KIND | DATE | WEEK | LA | PG |
|---|---|---|---|---|---|
| WO 9961055 | A1 | 19991202 | (200008)* | EN | 67 |
| RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ UG ZW | | | | | |
| W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG UZ VN YU ZA ZW | | | | | |
| AU 9940937 | A | 19991213 | (200020) | | |
| EP 1079859 | A1 | 20010307 | (200114) | EN | |
| R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE | | | | | |

APPLICATION DETAILS:

| PATENT NO | KIND | APPLICATION | DATE |
|---|---|---|---|
| WO 9961055 | A1 | WO 1999-US11296 | 19990521 |
| AU 9940937 | A | AU 1999-40937 | 19990521 |
| EP 1079859 | A1 | EP 1999-924431 | 19990521 |
| | | WO 1999-US11296 | 19990521 |

FILING DETAILS:

| PATENT NO | KIND | | PATENT NO |
|---|---|---|---|
| AU 9940937 | A | Based on | WO 9961055 |
| EP 1079859 | A1 | Based on | WO 9961055 |

PRIORITY APPLN. INFO: US 1998-86451 19980522
Searcher : Shears 308-4994

AN 2000-097072 [08] WPIDS
AB WO 9961055 A UPAB: 20000215
NOVELTY - Non-naturally occurring **bifunctional** molecule (I) of less than 5000 daltons, is new and consists of a drug moiety and a presenter protein **ligand** which are optionally joined by a **linking** group. The drug moiety has enhanced activity as compared to a free drug control.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a synthetic **bifunctional** molecule of less than about 5000 daltons with a formula Z-L-X, where X is a drug moiety, L is a bond or **linking** group, and Z is a **ligand** for an endogenous presenter protein **ligand**, the drug moiety has enhanced activity compared to a free drug compound;

(2) a method for producing a binary complex in a host comprising administering (I) to a host;

(3) a method for producing a tripartite complex in a mammalian host comprising administering (I) the **bifunctional** molecule binds to a drug target and presenter protein to produce tripartite complex;

(4) a method for enhancing the selectivity of a drug for a target in a first cell as compared to a second cell comprising contacting both cells with **bifunctional** molecule which comprises a drug and a **ligand** in the second cell but not in the first cell, where a binary complex which comprises a **bifunctional** molecule and presenter protein is produced in the second cell;

(5) a method of administering a drug to a host in need of such by administering an amount of a **bifunctional** molecule consisting a drug or its fragment covalently **linked**, directly or through a **linking** group, to a **ligand**
;

(6) a method of making a **bifunctional** molecule comprising

(a) identifying a drug moiety;

(b) preparing a library of **bifunctional** molecules where each shares a common **ligand** and drug moiety separated by a variable **linking**; and

(c) screening the library to identify members with enhanced activity compared to corresponding free drug;

(7) a pharmaceutical composition comprising a **bifunctional** molecule; and

(8) a kit comprising the composition of (7) and instructions for use in a therapeutic method.

ACTIVITY - Immunosuppressant; virucide.

MECHANISM OF ACTION - **Inhibitor**.

USE - **Bifunctional** molecules can be incorporated into pharmaceutical compositions which are used in the treatment of cellular proliferative diseases such as neoplastic, autoimmune,

cardiovascular, hormonal abnormality and infectious diseases.

ADVANTAGE - The **bifunctional** molecule binds to the presenter protein to produce the binary complex having enlarged target binding surface area such that enhanced affinity, specificity or selectivity are observed as compared to the free drug.

Dwg.0/14

L27 ANSWER 8 OF 29 MEDLINE DUPLICATE 3

ACCESSION NUMBER: 1999394991 MEDLINE

DOCUMENT NUMBER: 99394991

TITLE: Pharmacological inhibition of Ras-transformed epithelial cell growth is **linked** to down-regulation of epidermal growth factor-related peptides.

AUTHOR: Sizemore N; Cox A D; Barnard J A; Oldham S M; Reynolds E R; Der C J; Coffey R J

CORPORATE SOURCE: Departments of Medicine and Cell Biology, Vanderbilt University and Veterans Affairs Medical Center, Nashville, Tennessee, USA.

CONTRACT NUMBER: CA46413 (NCI)
DK49637 (NIDDK)
5T32 DK076 73-05 (NIDDK)
+

SOURCE: GASTROENTEROLOGY, (1999 Sep) 117 (3) 567-76.
Journal code: FH3. ISSN: 0016-5085.

PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals; Cancer Journals

ENTRY MONTH: 199911

AB BACKGROUND & AIMS: Posttranslational farnesylation is required for Ras activation. Farnesyl transferase **inhibitors** (FTIs) selectively **block protein** farnesylation and reduce the growth of many Ras-transformed cells in vitro and in vivo. Activated Ras transforms rat intestinal epithelial (RIE-1) cells by a mechanism distinct from NIH 3T3 fibroblasts in that an epidermal growth factor receptor (EGFR) autocrine loop contributes significantly to the Ras-transformed RIE-1 phenotype. METHODS: The ability of FTIs to block growth of Ras-transformed RIE-1 cells was evaluated, and these results were correlated with decreased EGFR **ligand** production. RESULTS: FTI L744,832 caused a selective, dose-dependent, reversible blockade in proliferation of H-Ras-transformed RIE-1 cells, whereas control cell lines, K-Ras-transformed cells, and activated raf-transfected RIE cells were unaffected. The growth-inhibitory effects of L744,832 correlated with loss of farnesylated H-Ras protein and a marked reduction in transforming growth factor (TGF)-alpha and amphiregulin expression. Inhibition of proliferation of H-Ras RIE-1 cells by

L744,832 was overcome by exogenous TGF-alpha, and enhanced growth inhibition was achieved by EGFR blockade in combination with L744,832. +CONCLUSIONS: These data suggest that one mechanism by which FTIs inhibit growth of H-Ras-transformed epithelial cells is by reducing Ras-induced EGFR **ligand** production.

L27 ANSWER 9 OF 29 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 4
ACCESSION NUMBER: 1999:312528 BIOSIS
DOCUMENT NUMBER: PREV199900312528
TITLE: The mechanism of growth-promoting effects of prolactin in embryogenesis - **Links** to growth factors.
AUTHOR(S): Karabulut, A. K.; Layfield, R.; Pratten, M. K. (1)
CORPORATE SOURCE: (1) Department of Human Anatomy and Cell Biology, Queens Medical Centre, Nottingham, NG7 2UH UK
SOURCE: Cells Tissues Organs, (1999) Vol. 164, No. 1, pp. 2-13.
ISSN: 1422-6405.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The polypeptide hormone prolactin (PRL) has been implicated in the regulation of embryonic growth and development, but the control mechanisms involved in the effects of the hormone are poorly understood. Several investigators suggested that there may be a possible **link** between the effects of PRL and insulin-like growth factors (IGFs). Recent studies have also shown that **ligand**-induced activation of PRL receptors leads to tyrosine phosphorylation of multiple **intracellular proteins**, and tyrosine kinase activation takes place in mediating the mitogenic action of PRL. In order to determine whether IGFs are involved in mediating the growth-promoting effect of PRL, rat embryos were culture in vitro for 48 h in whole rat serum and serum depleted of low molecular weight molecules (30 kD retenate) supplemented with rat PRL in the presence and absence of antisera against rat PRL, IGF I and IGF II. To investigate the effects of inhibiting the signal transduction of the PRL receptors, the embryoswere preincubated for 2 h in retenate in the presence of tyrosine kinase **inhibitors**, tyrphostin 47 and genistein, then rat PRL was added to the culture medium. Embryos cultured in retenate showed severe growth retardation, and the addition of rat PRL caused significant increase in growth and development of the embryos suggesting that embryos may be able to utilize maternally derived PRL during organogenesis. The presence of antiserum against rat PRL abolished the PRL-induced increase in development and antibodies against IGF I and II had a similar effect, suggesting that IGFs may be involved in the effect of the hormone. The 2-hour preincubation with genistein and tyrphostin also abolished the PRL-induced increase in development. These results indicate that

(FILE 'CAPLUS' ENTERED AT 14:54:02 ON 16 MAR 2001)

| | | | |
|---|---|---|---|
| L1 | 1782 | S | (EXTRACELL? OR EXTRA CELL?)(W)PROTEIN |
| L2 | 10 | S | L1 AND (BIFUNCT? OR BI FUNCT?) |
| L3 | 127 | S | L1 AND INHIBITOR |
| L4 | 14 | S | L3 AND (CONJUGAT? OR LINK?) |
| L5 | 1 | S | L4 AND LIGAND |
| L6 | 11 | S | L2 OR L5 |

* May contain dup. cites. Term omitted from prev. search

L6 ANSWER 1 OF 11 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1998:515871 CAPLUS

DOCUMENT NUMBER: 129:227452

TITLE: Amino acid substitutions in PilD, a **bifunctional** enzyme of Pseudomonas aeruginosa. Effect on leader peptidase and N-methyltransferase activities in vitro and in vivo

AUTHOR(S): Pepe, Jeffrey C.; Lory, Stephen

CORPORATE SOURCE: Department of Microbiology, School of Medicine, University of Washington, Seattle, WA, 98195, USA

SOURCE: J. Biol. Chem. (1998), 273(30), 19120-19129
CODEN: JBCHA3; ISSN: 0021-9258

PUBLISHER: American Society for Biochemistry and Molecular Biology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Subunits of type IV pili and a subset of proteins of the type II **extracellular protein** secretion app. undergo two consecutive post-translational modifications: leader peptide cleavage, followed by methylation of the newly created N-terminal amino acid. These two reactions are carried out by a single **bifunctional** enzyme encoded in Pseudomonas aeruginosa by the pilD gene. Properties of PilD mutants at positions Gly95 and/or Lys96, which were differentially affected in leader peptidase and N-methyltransferase function, were characterized. None of the single amino acid substitutions showed a significant alteration in their ability to cleave the prepilin leader peptide; however, two double mutants did exhibit a modest redn. in the efficiency of cleavage. In contrast, a significant decrease of N-methyltransferase activity was detected in PilD having substitutions at Gly95. Mutants with substitutions at position Lys96 showed a variable effect on N-methyltransferase activity with an apparent requirement for any charged amino acid at this position. Absence of N-methyltransferase activity did not appear to interfere with the ability of P. aeruginosa to assemble functional pili. Moreover, pilin monomers isolated from P. aeruginosa expressing PilD with Gly95 substitutions were not methylated. Although complete methylation does not appear to be absolutely required for pilus assembly in P. aeruginosa, this modification may be important for

pilus function in its natural habitat.

L6 ANSWER 2 OF 11 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1997:568031 CAPLUS
DOCUMENT NUMBER: 127:245314
TITLE: Interactions of the components of the general secretion pathway: role of Pseudomonas aeruginosa type IV pilin subunits in complex formation and **extracellular protein** secretion
AUTHOR(S): Lu, Hong-Mei; Motley, S. Timothy; Lory, Stephen
CORPORATE SOURCE: Department of Microbiology, School of Medicine, University of Washington, Seattle, WA, 98195, USA
SOURCE: Mol. Microbiol. (1997), 25(2), 247-259
CODEN: MOMIEE; ISSN: 0950-382X
PUBLISHER: Blackwell
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The general secretion pathway (GSP), found in a wide range of bacteria, is responsible for extracellular targeting of a subset of proteins from the periplasm. In P. aeruginosa, the GSP requires the participation of 12 proteins, of which XcpT, XcpU, XcpV, XcpW are homologues of PilA, the major subunit of type IV pili. The interaction between the pilin-like Xcp proteins was investigated using **bifunctional** crosslinking reagents. Crosslinking anal. of whole cells of wild-type P. aeruginosa, followed by immunoblot anal., revealed a 34-kDa XcpT-contg. complex. This complex was shown to consist of XcpT/PilA heterodimers. The role of PilA in the GSP was examd., using P. aeruginosa mutants in the pilA gene, or in rpoN, a gene regulating pilA expression. Each mutant showed a significant redn. in the efficiency of **extracellular protein** secretion, and this defect could be restored by expression of the cloned pilA gene in the mutant cells. The formation of the PilA/XcpT complex did not require XcpR or XcpQ, 2 other components of the secretion machinery, nor did it require the pilus biogenesis factors PilB and PilC. The dimeric XcpT/PilA complex was also formed in a pilD mutant, which lacks the leader peptidase enzyme, demonstrating that the leader peptide at the N-terminus or PilA or XcpT did not have to be removed for the dimerization to occur. XcpW and XcpU can also be cross-linked to form dimeric complexes with PilA. When expression of XcpT is increased, its homodimers, as well as XcpT/XcpW heterodimers, can be detected. An oligohistidine-tagged XcpT was shown to form stoichiometric complexes with PilA, and with XcpT, U, and W. These dimers were co-purified by nickel-affinity chromatog. The results of this study suggest that XcpT can form heterodimers with PilA, and with Xcp U, V and W, which may be assembly intermediates of the secretion app. Alternatively, these may

represent dynamic intermediates that facilitate protein secretion by continuous assocn. and dissocn. The requirement for PilA for efficient protein secretion argues for a crit. role played by PilA in 2 related processes during P. aeruginosa infections: formation of an adhesive pilus organelle and secretion of exoenzymes.

L6 ANSWER 3 OF 11 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1997:465233 CAPLUS
DOCUMENT NUMBER: 127:146315
TITLE: Structure-function relationship of type-IV prepilin peptidase of pseudomonas aeruginosa - a review
AUTHOR(S): Lory, Stephen; Strom, Mark S.
CORPORATE SOURCE: Dep. Microbiology, Univ. Washington, Seattle, WA, 98195, USA
SOURCE: Gene (1997), 192(1), 117-121
CODEN: GENED6; ISSN: 0378-1119
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review with 26 refs. The **bifunctional** enzyme prepilin peptidase (PilD) from Pseudomonas aeruginosa is a key determinant in both type-IV pilus biogenesis and **extracellular protein** secretion, in its roles as a leader peptidase and MTase. It is responsible for endopeptidic cleavage of th unique leader peptides that characterize type-Iv pilin precursors, as well as proteins with homologous leader sequences that are essential components of the general secretion pathway found in a variety of Gram-neg. pathogens. Following removal of the leader peptides, the same enzyme is responsible for the second posttranslational modification that characterizes the type-IV pilins and their homologues, namely N-methylation of the newly exposed N-terminal amino acid residue. This review discusses some of the work begun in order to answer questions regarding the structure-function relationships of the active sites of this unique enzyme.

L6 ANSWER 4 OF 11 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1997:336926 CAPLUS
DOCUMENT NUMBER: 126:340090
TITLE: The family of the small leucine-rich proteoglycans: key regulators of matrix assembly and cellular growth
AUTHOR(S): Iozzo, Renato V.
CORPORATE SOURCE: Department of Pathology, Anatomy and Cell Biology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA, 19107, USA
SOURCE: Crit. Rev. Biochem. Mol. Biol. (1997), 32(2), 141-174

CODEN: CRBBEJ; ISSN: 1040-9238
PUBLISHER: CRC
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review with 162 refs., on conceptual and functional advances in understanding small leucine-rich proteoglycans. These mols. belong to an expanding gene class whose distinctive feature is a structural motif, called the leucine-rich repeat, found in an increasing no. of intracellular and **extracellular proteins** with diverse biol. attributes. Three-dimensional modeling of their prototype protein core proposes a flexible, arch-shaped binding surface suitable for strong and distinctive interactions with ligand proteins. Changes in the properties of individual proteoglycans derive from amino acid substitutions in the less conserved surface residues, changes in the no. and length of the leucine-rich repeats, and/or variation in glycosylation. These proteoglycans are tissue organizers, orienting and ordering collagen fibrils during ontogeny and in pathol. processes such as wound healing, tissue repair, and tumor stroma formation. These properties are rooted in their **bifunctional** character: the protein moiety binding collagen fibrils at strategic loci, the microscopic gaps between staggered fibrils, and the highly charged glycosaminoglycans extending out to regulate interfibrillar distances and thereby establishing the exact topol. of fibrillar collagens in tissues. These proteoglycans also interact with sol. growth factors, modulate their functional activity, and bind to cell surface receptors. The latter interaction affects cell cycle progression in a variety of cellular systems and could explain the purported changes in the expression of these gene products around the invasive neoplastic cells and in regenerating tissues.

L6 ANSWER 5 OF 11 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1996:146310 CAPLUS
DOCUMENT NUMBER: 124:222297
TITLE: Cloning of an Aeromonas hydrophila type IV pilus biogenesis gene cluster: complementation of pilus assembly functions and characterization of a type IV leader peptidase/N-methyltransferase required for **extracellular protein** secretion
AUTHOR(S): Pepe, Cynthia M.; Eklund, Melvin W.; Strom, Mark S.
CORPORATE SOURCE: US Dep. Com., NOAA, Seattle, WA, 98112-2097, USA
SOURCE: Mol. Microbiol. (1996), 19(4), 857-69
CODEN: MOMIEE; ISSN: 0950-382X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Aeromonas hydrophila secretes several **extracellular proteins** that are assocd. with virulence including an

enterotoxin, a protease, and the hole-forming toxin, aerolysin. These degradative enzymes and toxins are exported by a conserved pathway found in many Gram-neg. bacteria. In Pseudomonas aeruginosa this export pathway and type IV pilus biogenesis are dependent on the product of the pilD gene. PilD is a **bifunctional** enzyme that processes components of the extracellular secretory pathway as well as a type IV prepilin. An A. hydrophila genomic library was transferred into a P. aeruginosa pilD mutant that is defective for type IV pilus biogenesis. The A. hydrophila pilD homolog, tapD, was identified by its ability to complement the pilD mutation in P. aeruginosa. Transconjugants contg. tapD were sensitive to the type IV pilus-specific phage, PO4. Sequence data revealed that tapD is part of a cluster of genes (tapABCD) that are homologous to P. aeruginosa type IV pilus biogenesis genes (pilABCD). TapB and TapC are functionally homologous to P. aeruginosa PilB and PilC, the first such functional complementation of pilus assembly demonstrated between bacteria that express type IV pili. In vitro studies revealed that TapD has both endopeptidase and N-methyltransferase activities using P. aeruginosa prepilin as substrate. Furthermore, tapD is required for extracellular secretion of aerolysin and protease, indicating that tapD may play an important role in the virulence of A. hydrophila.

L6 ANSWER 6 OF 11 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1994:4087 CAPLUS
DOCUMENT NUMBER: 120:4087
TITLE: Structure-function and biogenesis of the type IV pili
AUTHOR(S): Strom, Mark S.; Lory, Stephen
CORPORATE SOURCE: Dep. Microbiol., Univ. Washington, Seattle, WA, 98195, USA
SOURCE: Annu. Rev. Microbiol. (1993), 47, 565-96
CODEN: ARMIAZ; ISSN: 0066-4227
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review with 127 refs. Type IV pili are adhesins expressed by a no. of diverse gram-neg. microorganisms. These pili are related through similarities in the primary amino acid sequences of the structural subunits, a conserved assembly machinery, and a similar mechanism of transcriptional regulation. Type IV pilus assembly is preceded by proteolytic processing and N-methylation of the pilin polypeptide. This process is carried out by a novel **bifunctional** enzyme, PilD, first identified in Pseudomonas aeruginosa. Moreover, proteins homologous with type IV pilins have been shown to function in **extracellular protein** secretion in gram-neg. bacteria and in transformation competence in gram-pos. microorganisms. Like prepilin, these proteins are also processed and N-methylated by PilD. Transcription of the genes for type IV pilins is carried out by a RNA polymerase with a minor sigma

factor, RpoN. In P. aeruginosa two other regulatory elements (PilS and PilR) are required for pilin expression. RpoN, but not PilS and Pilr, is required for expression of a diverse set of bacterial genes. Therefore, regulation of synthesis and posttranslational modification and assembly of type IV pili serves as a useful model for a no. of diverse biol. processes in the bacterial cell.

L6 ANSWER 7 OF 11 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1993:555896 CAPLUS
DOCUMENT NUMBER: 119:155896
TITLE: Cleavage, methylation, and localization of the Pseudomonas aeruginosa export proteins XcpT, -U, -V, and -W
AUTHOR(S): Nunn, David N.; Lory, Stephen
CORPORATE SOURCE: Dep. Microbiol., Univ. Illinois, Urbana, IL, 61801, USA
SOURCE: J. Bacteriol. (1993), 175(14), 4375-82
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Four components of the app. of **extracellular protein** secretion of Pseudomonas aeruginosa, Xcpt, -U, -V, and -W (XcpT-W), are synthesized as precursors with short N-terminal leader peptides that share sequence similarity with the pilin subunit of this organism. A specialized leader peptidase/methylase, product of the pilD gene, has been shown to cleave the leader peptide from prepilin and to methylate the N-terminal phenylalanine of the mature pilin. Antibodies were prepd. against XcpT-W and used to purify each of these proteins. Sequence anal. of XcpT-W has shown that these proteins, like mature pilin, contain N-methylphenylalanine as the N-terminal amino acid. Anal. of cellular fractions from wild-type and pilD mutant strains of P. aeruginosa showed that the precursor forms of XcpT-W are located predominantly in the bacterial inner membrane, and their localization is not altered after PilD-mediated removal of the leader sequence. Evidently, the biogenesis of the app. of **extracellular protein** secretion and of type IV pili share a requirement for PilD. This **bifunctional** enzyme, acting in the inner membrane, cleaves the leader peptides from precursors of pilins and XcpT-W and subsequently methylates the amino group of the N-terminal phenylalanine of each of its substrates.

L6 ANSWER 8 OF 11 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1993:554829 CAPLUS
DOCUMENT NUMBER: 119:154829
TITLE: Identification of active-site cysteines in the conserved domain of PilD, the **bifunctional** type IV pilin leader

peptidase/N-methyltransferase of Pseudomonas aeruginosa
AUTHOR(S): Strom, Mark S.; Bergman, Phil; Lory, Stephen
CORPORATE SOURCE: Sch. Med., Univ. Washington, Seattle, WA, 98195, USA
SOURCE: J. Biol. Chem. (1993), 268(21), 15788-94
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB PilD is a **bifunctional** enzyme responsible for cleavage of the leader peptides from the precursors of the type IV pilin and four proteins with type IV pilin-like amino termini that are required for **extracellular protein** secretion in Pseudomonas aeruginosa. Following cleavage, PilD also catalyzes the second major post-translational modification of these proteins, namely the N-methylation of the amino-terminal phenylalanine residues of the mature polypeptides. In this report, it was demonstrated that the enzymic activities of PilD involve cysteine residues that lie within a cytoplasmic domain that shows a high degree of similarity to other proteins postulated to perform the same function in other bacterial species. Both activities are reduced in the presence of sulfhydryl-reactive reagents such as N-ethylmaleimide and p-chloromercuribenzoate. Mutagenesis of pilD resulting in specific amino acid substitutions in all of the Cys residues in PilD show that the 4 conserved cysteines in the cytoplasmic domain are required for full peptidase activity in vivo and for complete peptidase and methyltransferase activities in vitro. Conversely, substitution for a Cys residue in a membrane spanning domain had no effect on PilD activities in vivo or in vitro. Evidence suggests that the peptidase and methyltransferase sites of PilD are adjacent, with the Cys residues in the cytoplasmic domain important for Me donor binding, as prior reaction of PilD with the S-adenosyl-L-methionine analog sinefungin afforded complete protection of peptidase activity from inactivation with N-ethylmaleimide.

L6 ANSWER 9 OF 11 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1993:466578 CAPLUS
DOCUMENT NUMBER: 119:66578
TITLE: A single **bifunctional** enzyme, PilD, catalyzes cleavage and N-methylation of proteins belonging to the type IV pilin family
AUTHOR(S): Strom, Mark S.; Nunn, David N.; Lory, Stephen
CORPORATE SOURCE: Sch. Med., Univ. Washington, Seattle, WA, 98195, USA
SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1993), 90(6), 2404-8
CODEN: PNASA6; ISSN: 0027-8424
DOCUMENT TYPE: Journal

LANGUAGE: English

AB Precursors of the type IV pilins of a no. of bacterial pathogens, as well as related proteins involved in **extracellular protein** export and DNA uptake, were synthesized with short basic leader sequences. Maturation of these proteins involves 2 consecutive posttranslational modifications. The leader sequence is 1st proteolytically removed by specialized endopeptidases, of which the prototype is encoded by the pilD gene of Pseudomonas aeruginosa. Subsequently, the N-termini of these proteins are methylated. Here, it is demonstrated that PilD, in addn. to cleaving the N-terminal leader sequences of prepilin, also catalyzes the N-methylation of the N-terminal phenylalanine of the mature pilin, using S-adenosyl-L-methionine as Me donor. Thus, PilD is apparently the 1st characterized bacterial N-methyltransferase. Complete inhibition of N-methylation, but not peptide cleavage, by structural analogs of S-adenosyl-L-methionine suggested that PilD is a **bifunctional** enzyme with proteolytic and methylation activities carried out within 2 distinct active sites.

L6 ANSWER 10 OF 11 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1986:530457 CAPLUS

DOCUMENT NUMBER: 105:130457

TITLE: A comparative study of the extracellular glucosyl- and fructosyltransferases from cariogenic and non-cariogenic Streptococcus mutans strains of two different serotypes

AUTHOR(S): Asem, K.; Cornish-Bowden, A. J.; Cole, J. A.

CORPORATE SOURCE: Dep. Biochem., Univ. Birmingham, Birmingham, B15 2TT, UK

SOURCE: Microbios (1986), 47(190), 53-66
CODEN: MCBIA7; ISSN: 0026-2633

DOCUMENT TYPE: Journal

LANGUAGE: English

AB **Extracellular proteins** from continuous cultures of serotype c and g S. mutans strains were sepd. by polyacrylamide gel electrophoresis in the presence of SDS. Gels stained with raffinose after electrophoresis revealed that although serotype c strains secrete 2 fructosyltransferases of mol. mass 88 kilodaltons (kDa) and 79 kDa, no fructosyltransferase was secreted by the serotype g strain K1. A sucrose activity stain was used to detect 2 glucosyltransferases (gTF) of mol. mass 162 kDa ( **bifunctional** 1,6-.alpha.-D-glucan 3-.alpha.- and 6-.alpha. GTF or dextransucrase) and 153 kDa (a 1,3-.alpha.-D-glucan 3-.alpha.-GTF) in samples from cariogenic serotype c strains. Neither the 153 kDa protein nor the corresponding GTF activity was secreted by the noncarciogenic mutant C 67-25. The mol. masses of the corresponding 1,3-.alpha. and 1,6-.alpha.-GTF proteins from the serotype g strain K1 were 164 kDa and 158 kDa, resp. All of the GTF proteins were degraded to discrete bands of lower mol. mass on

storage at 4.degree. even after extensive purifn. The results provide an explanation for several outstanding controversies in the GTF literature.

L6 ANSWER 11 OF 11 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1981:136489 CAPLUS
DOCUMENT NUMBER: 94:136489
TITLE: Evidence for the involvement of transglutaminase in the uptake of vitellogenin by Xenopus laevis oocytes
AUTHOR(S): Tucciarone, Linda M.; Lanclos, Kenneth D.
CORPORATE SOURCE: Dep. Cell Mol. Biol., Med. Coll. Georgia, Augusta, GA, 30912, USA
SOURCE: Biochem. Biophys. Res. Commun. (1981), 99(1), 221-7
CODEN: BBRCA9; ISSN: 0006-291X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Vitellogenin is sequestered by the developing oocyte by receptor-mediated endocytosis, the process by which cells bind and internalize **extracellular proteins**. Endocytosis of a variety of proteins follows a similar pathway, whereby internalization of receptor-bound **ligand** takes place over clathrin-coated regions of the cell membrane. The protein cross-**linking** enzyme, transglutaminase (I), has been reported to be essential for the receptor-mediated endocytosis of insulin and .alpha.2-macroglobulin. I activity was demonstrated in the X. laevis ovary and was effectively inhibited by poly-L-lysine, an **inhibitor** of vitellogenin uptake, and dansylcadaverine, a known **inhibitor** of I activity. Two other less potent I **inhibitors**, MeNH2 and bacitracin, produced partial inhibition of ovarian I. Furthermore, dansylcadaverine and MeNH2 inhibited the appearance of vitellogenin in the yolk platelets of the oocyte.

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, JAPIO' ENTERED AT 14:56:39 ON 16 MAR 2001)
L7 49 S L6
L8 19 DUP REM L7 (30 DUPLICATES REMOVED)

L8 ANSWER 1 OF 19 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2001-032034 [04] WPIDS
DOC. NO. NON-CPI: N2001-025000
DOC. NO. CPI: C2001-009858
TITLE: Novel fragment complementation system to identify interactions between polypeptides comprises fragment pairs having first and second members that reassemble into a marker protein which has a directly detectable signal.
Searcher : Shears 308-4994

DERWENT CLASS: B04 D16 S03
INVENTOR(S): BALINT, R F; HER, J
PATENT ASSIGNEE(S): (PANO-N) PANORAMA RES INC
COUNTRY COUNT: 90
PATENT INFORMATION:

| PATENT NO | KIND | DATE | WEEK | LA | PG |
|---|---|---|---|---|---|
| WO 2000071702 | A1 | 20001130 | (200104)* | EN | 94 |

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW

APPLICATION DETAILS:

| PATENT NO | KIND | APPLICATION | DATE |
|---|---|---|---|
| WO 2000071702 | A1 | WO 2000-US7108 | 20000316 |

PRIORITY APPLN. INFO: US 2000-175968 20000113; US 1999-135926 19990525
AN 2001-032034 [04] WPIDS
AB WO 200071702 A UPAB: 20010118
NOVELTY - A fragment complementation system (I) which comprises a first oligopeptide (OP1) containing an N-terminal fragment with a C-terminal break point and a second oligopeptide (OP2) comprising a C-terminal with a N-terminal breakpoint, in which the C and N terminal fragments are both derived from a marker protein (MP) and reassemble to form a functionally reconstituted MP, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) identifying (M1) a functional fragment pair (FP) in a protein by preparing fragments of MP to obtain a marker fragment library (II), expressing members of (II) in many host cells, isolating host cells expressing MP as indicative of a cell containing first and second member of a FP which have formed a functionally reconstituted MP, by which the functional FP is identified. Each fragment of MP prepared has a break point terminus within a solvent exposed group of MP, in which the N or C terminal residue of each C or N terminal fragment, respectively constitutes the break point terminus to obtain (II);

(2) an expression cassette (III) comprising as operably **linked** components in the direction of transcription, nucleotide sequences encoding for:

(i) a promoter functional in a host cell, a polypeptide

interactor domain, FPL and C-terminal fragment of MP that provides for a selectable phenotype; or

(ii) a promoter functional in a host cell, an N-terminal fragment of a protein that provides for a selectable phenotype, FPL and a polypeptide interactor domain);

(3) identifying a OP2 to which a OP1 binds by:

(i) co-expressing in several host cells, OP1 and OP2 (which is a encoded by a member of a library) as a fusion protein with first and second members of FP of an MP, respectively, in which the binding of the OP1 to the OP2 results in functional reassembly of MP;

(ii) isolating host cells expressing the MP as indicative of a cell containing OP1 and OP2 which have interacted; and

(iii) sequencing plasmids containing expression cassettes coding for the fusion proteins, by which the OP2 to which the OP1 binds is identified; and

(4) host cells comprising expression cassettes (i) and (ii) of (2).

USE - The methods are used for monitoring the occurrence of protein-protein interactions in a sample, identifying oligopeptide interactions between two different proteomes, identifying epitopes that bind to an immunoglobulin (Ig) variable region, for identifying interactions between an extracellular domain of a transmembrane protein and a polypeptide, for high-throughput identification of compounds that inhibit phosphorylation-regulated signal transducers, forming a enzyme complementation system for selecting simultaneous incorporation of multiple genetic elements into a host cell and for activating a beta -lactam derivative of an antitumor compound in a host who is in need of it (all claimed).

(I) is used in human therapeutics, diagnostics and prognostics as well as in high-throughput systems for the discovery and validation of pharmaceutical targets and drugs. The interaction-activated enzyme association systems comprising prokaryotic beta -lactamase, are useful for simple and multiplex protein-protein interaction mapping, to enrich randomly primed expressed sequenced libraries for fragments which encode autonomously folding domains. It is also useful for interface mapping and **ligand** identification by mimotope homology, as bio-action sensors, in homogeneous assays and in target activated enzyme prodrug therapy (TAcEPT) and target-activate enzyme imaging (TAcEI). FP that comprise molecular interaction-dependent enzymes find use in homogeneous assays and biosensors for any analyte having two or more independent binding sites, tissue-localized activation of therapeutic and imaging reagents in vivo for early detection and treatment of cancer, chronic inflammation, atherosclerosis, amyloidosis, infection, transplant rejection, and other pathologies, cell-based sensors for activation or inhibition of metabolic or signal transduction pathways for high-efficiency, high-throughout screening for agonists/antagonists of the target pathway,

high-throughput mapping of pair-wise protein-protein interactions within and between the proteomes of cell, tissues, and pathogenic organisms, rapid selection of antibody fragments or other binding proteins which bind specifically to polypeptides of interest, rapid antigen identification for anti-cell and anti-tissue antibodies, rapid epitope identification for antibodies and in cell-based screens for high-throughput selection of **inhibitors** of any protein-protein interaction.

ADVANTAGE - Inclusion of a reporter protein in (I) provides for a directly detectable signal upon reassembly and background levels of 1 in 106. The system further provides for rationally incorporated enhancement modifications to the fusion oligopeptides that increase the functional activity of the reconstituted protein to wild type levels by improving folding and reassembly of the fragments into the parent protein while at the same time maintaining dependence on the interactor domains for reassembly.

DESCRIPTION OF DRAWING(S) - The figure shows a mechanism for interaction-dependent enzyme activation.

Dwg.1/11

L8 ANSWER 2 OF 19 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD

ACCESSION NUMBER: 2001-031651 [04] WPIDS
DOC. NO. NON-CPI: N2001-024806
DOC. NO. CPI: C2001-009575
TITLE: Treating implantable biological tissues such as prosthetic components by stabilizing glycosaminoglycans and crosslinking proteins on tissues to give improved mechanical integrity and in vivo stability.
DERWENT CLASS: A96 B04 D22 P32 P34
INVENTOR(S): LEVY, R J; VYAVAHARE, N
PATENT ASSIGNEE(S): (CHIL-N) CHILDRENS HOSPITAL PHILADELPHIA
COUNTRY COUNT: 19
PATENT INFORMATION:

| PATENT NO | KIND | DATE | WEEK | LA | PG |
|---|---|---|---|---|---|
| WO 2000064371 | A1 | 20001102 | (200104)* | EN | 20 |

RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE
W: CA

APPLICATION DETAILS:

| PATENT NO | KIND | APPLICATION | DATE |
|---|---|---|---|
| WO 2000064371 | A1 | WO 2000-US11289 | 20000426 |

PRIORITY APPLN. INFO: US 1999-131257 19990427
Searcher : Shears 308-4994

AN 2001-031651 [04] WPIDS
AB WO 200064371 A UPAB: 20010118
NOVELTY - Treating implantable biological tissues, comprising stabilizing glycosaminoglycans (GAGs) and crosslinking proteins on the tissue, is new.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a kit for pre-implantation treatment of biological tissues, comprising a reagent for stabilizing GAGS on the tissue, and a reagent for cross-linking proteins on the tissue.

USE - The methods are used to treat implantable biological tissues including those that form part of an implantable bioprosthetic device, such as heart valve prostheses, particularly porcine heart valves or bovine pericardium-derived heart valve prostheses, vascular grafts, skin grafts, dura mater grafts, cartilage grafts or implants, pericardium grafts, urinary bladder prostheses, ligament prostheses or tendon prostheses (claimed).

ADVANTAGE - The methods improve the mechanical integrity of the devices and improve their stability in vivo. They extend the useful life of bioprosthetic devices longer than is possible using prior-art fixation methods, potentially reducing the need for device replacement. They improve the durability of the tissue and its immune tolerance by the recipient.
Dwg.0/0

L8 ANSWER 3 OF 19 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 2000:71421 SCISEARCH
THE GENUINE ARTICLE: 275TQ
TITLE: The type 4 prepilin peptidases comprise a novel family of aspartic acid proteases
AUTHOR: LaPointe C F; Taylor R K (Reprint)
CORPORATE SOURCE: DARTMOUTH COLL SCH MED, DEPT MICROBIOL, HANOVER, NH 03755 (Reprint); DARTMOUTH COLL SCH MED, DEPT MICROBIOL, HANOVER, NH 03755
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (14 JAN 2000) Vol. 275, No. 2, pp. 1502-1510.
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814.
ISSN: 0021-9258.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 37
*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Type 4 prepilins or prepilin-like-proteins are secreted by a wide range of bacterial species and are required for a variety of functions including type 4 pilus formation, toxin and other enzyme secretion, gene transfer, and biofilm formation. A distinctive feature of these proteins is the presence of a specialized leader

peptide that is cleaved off by a cognate membrane-bound type 4 prepilin peptidase (TFPP) during the process of secretion. In this report we show that the TFPPs represent a novel family of bilobed aspartate proteases that is unlike any other protease. The active site pairs of aspartic acids of the two TFPPs in Vibrio cholerae are found at positions 125 and 189 of TcpJ and 147 and 212 of VcpD. Corresponding aspartate residues are completely conserved throughout this extensive peptidase family.

L8 ANSWER 4 OF 19 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 1998334648 MEDLINE
DOCUMENT NUMBER: 98334648
TITLE: Amino acid substitutions in PilD, a **bifunctional** enzyme of Pseudomonas aeruginosa. Effect on leader peptidase and N-methyltransferase activities in vitro and in vivo.
AUTHOR: Pepe J C; Lory S
CORPORATE SOURCE: Department of Microbiology, School of Medicine, University of Washington, Seattle, Washington 98195, USA.
CONTRACT NUMBER: ROI AI 21451 (NIAID)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Jul 24) 273 (30) 19120-9.
Journal code: HIV. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199810
AB Subunits of type IV pili and a subset of proteins of the type II **extracellular protein** secretion apparatus undergo two consecutive post-translational modifications: leader peptide cleavage, followed by methylation of the newly created N-terminal amino acid. These two reactions are carried out by a single **bifunctional** enzyme encoded in Pseudomonas aeruginosa by the pilD gene. Properties of PilD mutants at positions Gly95 and/or Lys96 which were differentially affected in leader peptidase and N-methyltransferase function were characterized. None of the single amino acid substitutions showed a significant alteration in their ability to cleave the prepilin leader peptide; however, two double mutants did exhibit a modest reduction in the efficiency of cleavage. In contrast, a significant decrease of N-methyltransferase activity was detected in PilD having substitutions at Gly95. Mutants with substitutions at position Lys96 showed a variable effect on N-methyltransferase activity with an apparent requirement for any charged amino acid at this position. Absence of N-methyltransferase activity did not appear to interfere with the ability of P. aeruginosa to assemble functional pili. Moreover, pilin monomers isolated from P. aeruginosa expressing PilD with Gly95 substitutions

were not methylated. Although complete methylation does not appear to be absolutely required for pilus assembly in P. aeruginosa, this modification may be important for pilus function in its natural habitat.

L8 ANSWER 5 OF 19 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 1998:754290 SCISEARCH
THE GENUINE ARTICLE: 123EK
TITLE: Characterization of a hemoglobin protease secreted by the pathogenic Escherichia coli strain EB1
AUTHOR: Otto B R (Reprint); vanDooren S J M; Nuijens J H; Luirink J; Oudega B
CORPORATE SOURCE: BIOCTR AMSTERDAM, INST MOL BIOL SCI, DEPT MOL MICROBIOL, DE BOELELAAN 1087, NL-1081 HV AMSTERDAM, NETHERLANDS (Reprint); PHARMING BV, NL-2333 CA LEIDEN, NETHERLANDS
COUNTRY OF AUTHOR: NETHERLANDS
SOURCE: JOURNAL OF EXPERIMENTAL MEDICINE, (21 SEP 1998) Vol. 188, No. 6, pp. 1091-1103.
Publisher: ROCKEFELLER UNIV PRESS, 1114 FIRST AVE, 4TH FL, NEW YORK, NY 10021.
ISSN: 0022-1007.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 48

*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Many pathogenic bacteria can use heme compounds as a source of iron. Pathogenic Escherichia coli strains are capable of using hemoglobin as an iron source. However, the mechanism of heme acquisition from hemoglobin is not understood for this microorganism. We present the first molecular characterization of a hemoglobin protease (Hbp) from a human pathogenic E. coli strain. The enzyme also appeared to be a heme-binding protein. Affinity purification of this **bifunctional** protein enabled us to identify the extracellular gene product, and to clone and analyze its gene. A purification procedure developed for Hbp allowed us to perform functional studies. The protein interacted with hemoglobin, degraded it and subsequently bound the released heme. These results suggest that the protein is involved in heme acquisition by this human pathogen. Hbp belongs to the so-called IgA1 protease-like proteins, as indicated by the kinetics of its membrane transfer and DNA sequence similarity. The gene of this protein appears to be located on the large pColV-K30 episome, that only has been isolated from human and animal pathogens. All these characteristics indicate that Hbp may be an important virulence factor that may play a significant role in the pathogenesis of E. coli infections.

L8 ANSWER 6 OF 19 MEDLINE DUPLICATE 2

ACCESSION NUMBER: 97426033 MEDLINE
DOCUMENT NUMBER: 97426033
TITLE: Interactions of the components of the general secretion pathway: role of Pseudomonas aeruginosa type IV pilin subunits in complex formation and **extracellular protein** secretion.
AUTHOR: Lu H M; Motley S T; Lory S
CORPORATE SOURCE: Department of Microbiology, School of Medicine, University of Washington, Seattle 98195, USA.
CONTRACT NUMBER: AI 21495 (NIAID)
SOURCE: MOLECULAR MICROBIOLOGY, (1997 Jul) 25 (2) 247-59.
Journal code: MOM. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199803
AB The general secretion pathway (GSP), found in a wide range of bacteria, is responsible for extracellular targeting of a subset of proteins from the periplasm. In Pseudomonas aeruginosa, the GSP requires the participation of 12 proteins, of which XcpT, XcpU, XcpV, XcpW are homologues of PilA, the major subunit of type IV pili. The interaction between the pilin-like Xcp proteins was investigated using **bifunctional** crosslinking reagents. Cross-linking analysis of whole cells of wild-type P. aeruginosa, followed by immunoblot analysis, revealed a 34-kDa XcpT-containing complex. This complex was shown to consist of XcpT/PilA heterodimers. The role of PilA in the GSP was examined, using P. aeruginosa mutants in the pilA gene, or in rpoN, a gene regulating pilA expression. Each mutant showed a significant reduction in the efficiency of **extracellular protein** secretion, and this defect could be restored by expression of the cloned pilA gene in the mutant cells. The formation of the PilA/XcpT complex did not require XcpR or XcpQ, two other components of the secretion machinery, nor did it require the pilus biogenesis factors PilB and PIlC. The dimeric XcpT/PilA complex was also formed in a pilD mutant, which lacks the leader peptidase enzyme, demonstrating that the leader peptide at the N-terminus or PilA or XcpT did not have to be removed for the dimerization to occur. XcpW and XcpU can also be crosslinked to form dimeric complexes with PilA. When expression of XcpT is increased, its homodimers, as well as XcpT/XcpW heterodimers, can be detected. Finally, an oligohistidine-tagged XcpT was shown to form stoichiometric complexes with PilA, and with XcpT, U, V and W. These dimers were co-purified by nickel-affinity chromatography. The results of this study suggest that XcpT can form heterodimers with PilA, and Xcp U, V and W, which may be assembly intermediates of the secretion apparatus. Alternatively, these may represent dynamic intermediates that facilitate protein secretion by continuous association and dissociation. The requirement for PilA

for efficient protein secretion argues for a critical role played by PilA in two related processes during P. aeruginosa infections: formation of an adhesive pilus organelle and secretion of exoenzymes.

L8 ANSWER 7 OF 19 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 97290640 MEDLINE
DOCUMENT NUMBER: 97290640
TITLE: The family of the small leucine-rich proteoglycans: key regulators of matrix assembly and cellular growth.
AUTHOR: Iozzo R V
CORPORATE SOURCE: Department of Pathology, Anatomy and Cell Biology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA 19107, USA.
CONTRACT NUMBER: RO1 CA39481-13 (NCI)
RO1 CA47282-07 (NCI)
SOURCE: CRITICAL REVIEWS IN BIOCHEMISTRY AND MOLECULAR BIOLOGY, (1997) 32 (2) 141-74. Ref: 162
Journal code: DTM. ISSN: 1040-9238.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199709
ENTRY WEEK: 19970901

AB The focus of this review is on conceptual and functional advances in our understanding of the small leucine-rich proteoglycans. These molecules belong to an expanding gene class whose distinctive feature is a structural motif, called the leucine-rich repeat, found in an increasing number of intracellular and **extracellular proteins** with diverse biological attributes.
Three-dimensional modeling of their prototype protein core proposes a flexible, arch-shaped binding surface suitable for strong and distinctive interactions with ligand proteins. Changes in the properties of individual proteoglycans derive from amino acid substitutions in the less conserved surface residues, changes in the number and length of the leucine-rich repeats, and/or variation in glycosylation. These proteoglycans are tissue organizers, orienting and ordering collagen fibrils during ontogeny and in pathological processes such as wound healing, tissue repair, and tumor stroma formation. These properties are rooted in their **bifunctional** character: the protein moiety binding collagen fibrils at strategic loci, the microscopic gaps between staggered fibrils, and the highly charged glycosaminoglycans extending out to regulate interfibrillar distances and thereby establishing the exact topology of fibrillar collagens in tissues. These proteoglycans also interact with soluble

growth factors, modulate their functional activity, and bind to cell surface receptors. The latter interaction affects cell cycle progression in a variety of cellular systems and could explain the purported changes in the expression of these gene products around the invasive neoplastic cells and in regenerating tissues.

L8 ANSWER 8 OF 19 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 97368114 MEDLINE
DOCUMENT NUMBER: 97368114
TITLE: Structure-function relationship of type-IV prepilin peptidase of Pseudomonas aeruginosa--a review.
AUTHOR: Lory S; Strom M S
CORPORATE SOURCE: Department of Microbiology, University of Washington, Seattle 98195, USA.. guidos@u.washington.edu
CONTRACT NUMBER: AI 21451 (NIAID)
SOURCE: GENE, (1997 Jun 11) 192 (1) 117-21. Ref: 26
Journal code: FOP. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-M32066; GENBANK-U20255; GENBANK-U48808; GENBANK-U17138; GENBANK-U32588; GENBANK-X74276; GENBANK-X70049; GENBANK-M32613; GENBANK-M24118; GENBANK-X14954; GENBANK-M74708; GENBANK-Z68186; GENBANK-M30805; GENBANK-U12432; GENBANK-L44941; GENBANK-L42023
ENTRY MONTH: 199710
AB The **bifunctional** enzyme prepilin peptidase (PilD) from Pseudomonas aeruginosa is a key determinant in both type-IV pilus biogenesis and **extracellular protein** secretion, in its roles as a leader peptidase and MTase. It is responsible for endopeptidic cleavage of the unique leader peptides that characterize type-IV pilin precursors, as well as proteins with homologous leader sequences that are essential components of the general secretion pathway found in a variety of Gram-negative pathogens. Following removal of the leader peptides, the same enzyme is responsible for the second posttranslational modification that characterizes the type-IV pilins and their homologues, namely N-methylation of the newly exposed N-terminal amino acid residue. This review discusses some of the work begun in order to answer questions regarding the structure-function relationships of the active sites of this unique enzyme.

L8 ANSWER 9 OF 19 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 96417863 MEDLINE
DOCUMENT NUMBER: 96417863

TITLE: Cloning of an Aeromonas hydrophila type IV pilus biogenesis gene cluster: complementation of pilus assembly functions and characterization of a type IV leader peptidase/N-methyltransferase required for **extracellular protein** secretion.

AUTHOR: Pepe C M; Eklund M W; Strom M S
CORPORATE SOURCE: US Department of Commerce, NOAA, Seattle, Washington 98112-2097, USA.

SOURCE: MOLECULAR MICROBIOLOGY, (1996 Feb) 19 (4) 857-69.
Journal code: MOM. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U20255
ENTRY MONTH: 199702

AB Aeromonas hydrophila secretes several **extracellular proteins** that are associated with virulence including an enterotoxin, a protease, and the hole-forming toxin, aerolysin. These degradative enzymes and toxins are exported by a conserved pathway found in many Gram-negative bacteria. In Pseudomonas aeruginosa this export pathway and type IV pilus biogenesis are dependent on the product of the pilD gene. PilD is a **bifunctional** enzyme that processes components of the extracellular secretory pathway as well as a type IV prepilin. An A. hydrophila genomic library was transferred into a P. aeruginosa pilD mutant that is defective for type IV pilus biogenesis. The A. hydrophila pilD homologue, tapD, was identified by its ability to complement the pilD mutation in P. aeruginosa. Transconjugants containing tapD were sensitive to the type IV pilus-specific phage, PO4. Sequence data revealed that tapD is part of a cluster of genes (tapABCD) that are homologous to P. aeruginosa type IV pilus biogenesis genes (pilABCD). We showed that TapB and TapC are functionally homologous to P. aeruginosa PilB and PilC, the first such functional complementation of pilus assembly demonstrated between bacteria that express type IV pili. In vitro studies revealed that TapD has both endopeptidase and N-methyltransferase activities using P. aeruginosa prepilin as substrate. Furthermore, we show that tapD is required for extracellular secretion of aerolysin and protease, indicating that tapD may play an important role in the virulence of A. hydrophila.

L8 ANSWER 10 OF 19 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 95292396 MEDLINE
DOCUMENT NUMBER: 95292396
TITLE: Galactosylation of N- and O-**linked** carbohydrate moieties of IgA1 and IgG in IgA nephropathy.
AUTHOR: Allen A C; Harper S J; Feehally J

CORPORATE SOURCE: Department of Nephrology, Leicester General Hospital, UK..
SOURCE: CLINICAL AND EXPERIMENTAL IMMUNOLOGY, (1995 Jun) 100 (3) 470-4.
Journal code: DD7. ISSN: 0009-9104.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199509
AB The mechanism of IgA deposition in the kidneys in IgA nephropathy is unknown. Mesangial IgA is of the IgA1 subclass, and since no consistent antigenic target for the IgA1 has been described, we have investigated the glycosylation of the molecule, as a potential non-immunological abnormality which may contribute to its deposition. IgA1 is rich in carbohydrate, carrying N-**linked** moieties in common with IgG, but also O-**linked** sugars, which are rare in serum proteins, and not expressed by IgG or IgA2. Lectin binding assays were designed to examine the expression of terminal galactose on the N-**linked** carbohydrate chains of purified serum IgG and IgA1, and the O-**linked** sugars of IgA1 and C1 **inhibitor** (one of the very few other serum proteins with O-**linked** glycosylation). No evidence was found for abnormalities of N-**linked** glycosylation of either isotype in IgA nephropathy compared with matched controls. However, in IgA nephropathy, reduced terminal galactosylation of the hinge region O-**linked** moieties was demonstrated; this was not seen in C1 **inhibitor**, which showed normal or increased galactosylation of the O-**linked** sugars. This abnormality of IgA1 has considerable implications for the pathogenesis of IgA nephropathy, since the O-**linked** sugars lie in an important functional location within the IgA1 molecule, close to the **ligand** of Fc receptors. Changes in the carbohydrates in this site may therefore affect interactions with receptors and **extracellular proteins**, leading to anomalous handling of the IgA1 protein in this condition, including failure of normal clearance mechanisms, and mesangial deposition.

L8 ANSWER 11 OF 19 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 94335721 MEDLINE
DOCUMENT NUMBER: 94335721
TITLE: Posttranslational processing of type IV prepilin and homologs by PilD of Pseudomonas aeruginosa.
AUTHOR: Strom M S; Nunn D N; Lory S
CORPORATE SOURCE: Utilization Research Division, Northwest Fisheries Science Center, NMFS, NOAA, Seattle, Washington 98112.
CONTRACT NUMBER: AI21451 (NIAID)
SOURCE: METHODS IN ENZYMOLOGY, (1994) 235 527-40. Ref: 30

Journal code: MVA. ISSN: 0076-6879.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199411

AB We have described the characterization of a protein initially identified as having an essential function in biogenesis of polar pili of P. aeruginosa by processing precursors of pilin. Other findings have also expanded the range of substrates for PilD to include a set of proteins that are essential components of the extracellular secretion machinery. Direct demonstration of prepilin processing necessitates use of purified substrates and enzymes, and we present general protocols for purification of both enzymes and substrates, as well as an assay for prepilin peptidase activity. For a source of enzyme and substrates, mutants of P. aeruginosa defective in pilin processing as well as clones overexpressing the pilin gene and PilD were developed. These methods are applicable to other bacterial systems that express Type IV pili and/or possess the PilD-dependent machinery of **extracellular protein** secretion. PilD is a **bifunctional** enzyme, which carries out not only cleavage but also amino-terminal methylation of the mature pilin. Cleavage and N-methylation of the pilin-like Xcp proteins involved in **extracellular protein** secretion have also been shown to be dependent on PilD. The leader peptidase activity of PilD is inhibited by sulfhydryl blocking reagents such as NEM and PCMB, whereas the methyltransferase activity of the purified enzyme is dependent on reduction with dithiothreitol. The conserved region containing the cysteine residues lies within the largest hydrophilic domain of the protein as predicted from hydrophobicity analysis, and it is probably exposed to the cytoplasmic side of the cytoplasmic membrane. Identification of the active site residues involved in recognition of the substrates for processing and subsequent methylation is currently underway. Studies on substrate specificities of PilD, with respect to its leader peptidase and methyltransferase activity, may prove to be useful in designing inhibitors which would interfere with maturation of Type IV prepilins and components of the **extracellular protein** secretion machinery. In light of the fact that an increasing number of both mammalian and plant pathogens are being shown to have extracellular secretion pathways homologous to that seen for P. aeruginosa, such inhibitors may be useful tools in the study of the role these peptidases play in bacterial virulence.

L8 ANSWER 12 OF 19 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 93340186 MEDLINE
Searcher : Shears 308-4994

DOCUMENT NUMBER: 93340186
TITLE: Identification of active-site cysteines in the conserved domain of PilD, the **bifunctional** type IV pilin leader peptidase/N-methyltransferase of Pseudomonas aeruginosa.
AUTHOR: Strom M S; Bergman P; Lory S
CORPORATE SOURCE: Department of Microbiology, School of Medicine, University of Washington, Seattle 98195.
CONTRACT NUMBER: AI21451 (NIAID)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1993 Jul 25) 268 (21) 15788-94.
Journal code: HIV. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199311

AB PilD is a **bifunctional** enzyme responsible for cleavage of the leader peptides from the precursors of the type IV pilin and four proteins with type IV pilin-like amino termini that are required for **extracellular protein** secretion in Pseudomonas aeruginosa. Following cleavage, PilD also catalyzes the second major posttranslational modification of these proteins, namely the N-methylation of the amino-terminal phenylalanine residues of the mature polypeptides. In this report, we demonstrate that the enzymatic activities of PilD involve cysteine residues that lie within a cytoplasmic domain that shows a high degree of similarity to other proteins postulated to perform the same function in other bacterial species. Both activities are reduced in the presence of sulfhydryl-reactive reagents such as N-ethylmaleimide and p-chloromercuribenzoate. Mutagenesis of pilD resulting in specific amino acid substitutions in all of the Cys residues in PilD show that the 4 conserved cysteines in the cytoplasmic domain are required for full peptidase activity in vivo and for complete peptidase and methyltransferase activities in vitro. Conversely, substitution for a Cys residue in a membrane spanning domain had no effect on PilD activities in vivo or in vitro. Evidence suggests that the peptidase and methyltransferase sites of PilD are adjacent, with the Cys residues in the cytoplasmic domain important for methyl donor binding, as prior reaction of PilD with the S-adenosyl-L-methionine analogue sinefungin afforded complete protection of peptidase activity from inactivation with N-ethylmaleimide.

L8 ANSWER 13 OF 19 MEDLINE DUPLICATE 9
ACCESSION NUMBER: 93322315 MEDLINE
DOCUMENT NUMBER: 93322315
TITLE: Cleavage, methylation, and localization of the Pseudomonas aeruginosa export proteins XcpT, -U, -V,

and -W.
AUTHOR: Nunn D N; Lory S
CORPORATE SOURCE: Department of Microbiology, University of Illinois, Urbana 61801.
CONTRACT NUMBER: AI32143 (NIAID)
AI21451 (NIAID)
SOURCE: JOURNAL OF BACTERIOLOGY, (1993 Jul) 175 (14) 4375-82.
Journal code: HH3. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199310
AB Four components of the apparatus of **extracellular protein** secretion of Pseudomonas aeruginosa, Xcpt, -U, -V, and -W (XcpT-W), are synthesized as precursors with short N-terminal leader peptides that share sequence similarity with the pilin subunit of this organism. A specialized leader peptidase/methylase, product of the pilD gene, has been shown to cleave the leader peptide from prepilin and to methylate the N-terminal phenylalanine of the mature pilin. Antibodies were prepared against XcpT-W and used to purify each of these proteins. Sequence analysis of XcpT-W has shown that these proteins, like mature pilin, contain N-methylphenylalanine as the N-terminal amino acid. Analysis of cellular fractions from wild-type and pilD mutant strains of P. aeruginosa showed that the precursor forms of XcpT-W are located predominantly in the bacterial inner membrane, and their localization is not altered after PilD-mediated removal of the leader sequence. These studies demonstrate that the biogenesis of the apparatus of **extracellular protein** secretion and that of type IV pili share a requirement for PilD. This **bifunctional** enzyme, acting in the inner membrane, cleaves the leader peptides from precursors of pilins and XcpT-W and subsequently methylates the amino group of the N-terminal phenylalanine of each of its substrates.

L8 ANSWER 14 OF 19 MEDLINE DUPLICATE 10
ACCESSION NUMBER: 93211970 MEDLINE
DOCUMENT NUMBER: 93211970
TITLE: A single **bifunctional** enzyme, PilD, catalyzes cleavage and N-methylation of proteins belonging to the type IV pilin family.
AUTHOR: Strom M S; Nunn D N; Lory S
CORPORATE SOURCE: Department of Microbiology, School of Medicine, University of Washington, Seattle 98195.
CONTRACT NUMBER: AI21451 (NIAID)
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1993 Mar 15) 90 (6) 2404-8.

Journal code: PV3. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199307

AB Precursors of the type IV pilins of a number of bacterial pathogens, as well as related proteins involved in **extracellular protein** export and DNA uptake, are synthesized with short basic leader sequences. Maturation of these proteins involves two consecutive posttranslational modifications. The leader sequence is first proteolytically removed by specialized endopeptidases, of which the prototype is encoded by the pilD gene of Pseudomonas aeruginosa. Subsequently, the amino termini of these proteins are methylated. Here we demonstrate that PilD, in addition to cleaving the amino-terminal leader sequences of prepilin, also catalyzes N-methylation of the amino-terminal phenylalanine of the mature pilin, using S-adenosyl-L-methionine as a methyl donor. Thus, to our knowledge, PilD is the first characterized bacterial N-methyltransferase. Complete inhibition of N-methylation, but not peptide cleavage, by structural analogues of S-adenosyl-L-methionine suggests that PilD is a **bifunctional** enzyme with proteolytic and methylation activities carried out within two distinct active sites.

L8 ANSWER 15 OF 19 MEDLINE DUPLICATE 11
ACCESSION NUMBER: 94079328 MEDLINE
DOCUMENT NUMBER: 94079328
TITLE: Structure-function and biogenesis of the type IV pili.
AUTHOR: Strom M S; Lory S
CORPORATE SOURCE: Department of Microbiology, University of Washington, Seattle 98195..
CONTRACT NUMBER: AI21451 (NIAID)
AI32624 (NIAID)
SOURCE: ANNUAL REVIEW OF MICROBIOLOGY, (1993) 47 565-96.
Ref: 127
Journal code: 6DV. ISSN: 0066-4227.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199403

AB Type IV pili are adhesins expressed by a number of diverse gram-negative microorganisms. These pili are related through similarities in the primary amino acid sequences of the structural subunits, a conserved assembly machinery, and a similar mechanism of

transcriptional regulation. Type IV pilus assembly is preceded by proteolytic processing and N-methylation of the pilin polypeptide. This process is carried out by a novel **bifunctional** enzyme PilD, first identified in Pseudomonas aeruginosa. Moreover, proteins homologous with type IV pilins have been shown to function in **extracellular protein** secretion in gram-negative bacteria and in transformation competence in gram-positive microorganisms. Like prepilin, these proteins are also processed and N-methylated by PilD. Transcription of the genes for type IV pilins is carried out by an RNA polymerase with a minor sigma factor, RpoN. In P. aeruginosa two other regulatory elements (PilS and PilR) are required for pilin expression. RpoN, but not PilS and PilR, is required for expression of a diverse set of bacterial genes. Therefore, regulation of synthesis and posttranslational modification and assembly of type IV pili serves as a useful model for a number of diverse biological processes in the bacterial cell.

L8 ANSWER 16 OF 19 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 92:507700 SCISEARCH
THE GENUINE ARTICLE: JK248
TITLE: SECRETION ACROSS THE BACTERIAL OUTER-MEMBRANE
AUTHOR: WANDERSMAN C (Reprint)
CORPORATE SOURCE: INST PASTEUR, UNITE GENET MOLEC, CNRS, UA 1149, 23 RUE DR ROUX, F-75724 PARIS 15, FRANCE (Reprint)
COUNTRY OF AUTHOR: FRANCE
SOURCE: TRENDS IN GENETICS, (SEP 1992) Vol. 8, No. 9, pp. 317-322.
ISSN: 0168-9525.
DOCUMENT TYPE: General Review; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 43
*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Many bacteria secrete **extracellular proteins** such as hydrolytic enzymes or toxins. In Gram-negative bacteria, secreted proteins must cross the two membranes that constitute the cell envelope. Recent studies have identified several specific secretion systems that can be classified in three distinct pathways, and related systems have been discovered in a wide range of prokaryotic and eukaryotic cells.

L8 ANSWER 17 OF 19 MEDLINE DUPLICATE 12
ACCESSION NUMBER: 87014128 MEDLINE
DOCUMENT NUMBER: 87014128
TITLE: A comparative study of the extracellular glucosyl- and fructosyltransferases from cariogenic and non-cariogenic Streptococcus mutans strains of two different serotypes.
AUTHOR: Asem K; Cornish-Bowden A J; Cole J A
Searcher : Shears 308-4994

SOURCE: MICROBIOS, (1986) 47 (190) 53-66.
Journal code: MXS. ISSN: 0026-2633.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198701

AB **Extracellular proteins** from continuous cultures of serotype c and g Streptococcus mutans strains were separated by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate. Gels stained with raffinose after electrophoresis revealed that although serotype c strains secrete two fructosyltransferases of molecular mass 68 kDa and 79 kDa, no fructosyltransferase was secreted by the serotype g strain K1. A sucrose activity stain was used to detect two glucosyltransferases (GTF) of molecular mass 162 kDa (**bifunctional** 1,6-alpha-D-glucan 3-alpha- and 6-alpha GTF or 'dextransucrase') and 153 kDa (a 1,3-alpha-D-glucan 3-alpha-GTF) in samples from cariogenic serotype c strains. Neither the 153 kDa protein nor the corresponding GTF activity was secreted by the non-cariogenic mutant C 67-25. The molecular masses of the corresponding 1,3-alpha and 1,6-alpha-GTF proteins from the serotype g strain K1 were 164 kDa and 158 kDa, respectively. All of the GTF proteins were degraded to discrete bands of lower molecular mass on storage at 4 degrees C even after extensive purification. The results provide an explanation for several outstanding controversies in the GTF literature.

L8 ANSWER 18 OF 19 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1984-245159 [40] WPIDS
DOC. NO. CPI: C1984-103487
TITLE: Bacillus subtilis mutant - useful for facilitating gene cloning to give stable transformants for **extracellular protein** secretion.
DERWENT CLASS: B04 D16
INVENTOR(S): OSTROOF, G R; PENE, J J
PATENT ASSIGNEE(S): (UYDE) UNIV DELAWARE
COUNTRY COUNT: 13
PATENT INFORMATION:

| PATENT NO | KIND | DATE | WEEK | LA | PG |
|---|---|---|---|---|---|
| EP 120629 | A | 19841003 | (198440)* | EN | 20 |
| R: AT BE CH DE FR GB IT LI LU NL SE | | | | | |
| JP 59224686 | A | 19841217 | (198505) | | |
| US 4595660 | A | 19860617 | (198627) | | |

APPLICATION DETAILS:

| PATENT NO | KIND | APPLICATION | DATE |
|---|---|---|---|

| | | | |
|---|---|---|---|
| EP 120629 | A | EP 1984-301405 | 19840302 |
| JP 59224686 | A | JP 1984-39695 | 19840301 |
| US 4595660 | A | US 1983-471789 | 19830302 |

PRIORITY APPLN. INFO: US 1983-471789 19830302
AN 1984-245159 [40] WPIDS
AB EP 120629 A UPAB: 19930925
Mutant of a restriction deficient strain of Bacillus subtilis having a phenotype for stable transformation by recombinant **bifunctional** plasmids propagated in Escherichia coli. It also has a reduced tendency, compared with the parent restriction deficient strain, to degrade E. coli-propagated chimeric plasmid DNA. Recombinant or chimeric **bifunctional** monomeric plasmid that replicates in both E. coli and B. subtilis and confers resistance to ampicillin and tetracycline in E. coli and to chloramphenical in B. subtilis. It contains a first DNA region capable of conferring resistance to ampicillin and tetracycline, a second DNA region conferring resistance to chloramphenicol and a third DNA region comprising a cloned inert contg. bacteriophage Phi 29 Hind III fragments.

USE/ADVANTAGE - The mutant is capable of stable transformation by clone pools or by individual recombinant **bifunctional** plasmids propagated in E. coli. The transformants are useful in biosynthetic processes as **extracellular protein** secretors.

0/0

ABEQ US 4595660 A UPAB: 19930925
A biologically pure culture of a spontaneous mutant of the MI 112 strain of Bacillus substilis (BS) being identified by the Bciluus Genetic Stock Centre collection no BGSC 1A510 is pref. used to produce a protein foreign to the BS by (A) transforming BS with a recombinant plasmid contg a gene encoding for the protein and (B) culturing the transformed mutant under conditions suitable for the expression of the protein and isolating the protein from the culture.

The spontaneous mutant is pref. obtd by (a) propagating a recombinant or chimeric **bifunctional** plasmid in E coli, which has a 1st DNA region able to confer a 1st antibiotic resistance in E coli, a 2nd DNA region able to confer a 2nd antibiotic resistance in BS and a 3rd DNA region comprising a cloned insert, (b) transforming the BS strain with the plasmid to obtain BS MI 112 composed mainly of 2nd antibiotic resistant, unstable transformants and a smaller amount of 2nd antibiotic resistant, virtually stable transformants contg intact the 3rd DNA region and including a spontaneous mutant (c) separating the lesser from the greater amt and curing the lesser amount by growing a multiplicity of generations without selective pressure from an antibiotic so that

later generations lose the above characteristic and (d) selecting the spontaneous mutant from the later generations.

USE/ADVANTAGE - For use in biosynthetic methods; a simpler and commercially more viable method yielding a protein low in or free from pyrogens.

L8 ANSWER 19 OF 19 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1981:235572 BIOSIS
DOCUMENT NUMBER: BA72:20556
TITLE: EVIDENCE FOR THE INVOLVEMENT OF TRANS GLUTAMINASE IN THE UPTAKE OF VITELLOGENIN BY XENOPUS-LAEVIS OOCYTES.
AUTHOR(S): TUCCIARONE L M; LANCLOS K D
CORPORATE SOURCE: DEP. CELL MOL. BIOL., MED. COLL. GA., AUGUSTA, GA. 30912.
SOURCE: BIOCHEM BIOPHYS RES COMMUN, (1981) 99 (1), 221-227. CODEN: BBRCA9. ISSN: 0006-291X.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB The yolk protein, vitellogenin, is sequestered by the developing oocyte by receptor-mediated endocytosis, the process by which cells bind and internalize **extracellular proteins**. Endocytosis of a variety of proteins follows a similar pathway, whereby internalization of receptor-bound **ligand** takes place over clathrin-coated regions of the cell membrane. The protein cross-**linking** enzyme, transglutaminase, was reportedly essential for the receptor-mediated endocytosis of insulin and .alpha.2-macroglobulin. The presence of transglutaminase activity was demonstrated in the X. laevis ovary and was effectively inhibited by poly L-lysine, an **inhibitor** of vitellogenin uptake, and dansylcadaverine, a known **inhibitor** of transglutaminase activity. Two other less potent **inhibitors** of transglutaminase, methylamine and bacitracin produced partial inhibition of the ovarian enzyme. Dansylcadaverine and methylamine inhibited the appearance of vitellogenin in the yolk platelets of the oocyte.

=> fil hom

FILE 'HOME' ENTERED AT 14:59:22 ON 16 MAR 2001

functional PRL receptors are present in rat embryos at this stage which may play an important role in the control of growth and development and this may be **linked** to growth factors and their receptors.

L27 ANSWER 10 OF 29 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 1998451442 MEDLINE
DOCUMENT NUMBER: 98451442
TITLE: The regulation of CD95 **ligand** expression and function in CTL.
AUTHOR: Li J H; Rosen D; Ronen D; Behrens C K; Krammer P H; Clark W R; Berke G
CORPORATE SOURCE: Department of Immunology, Weizmann Institute of Science, Rehovot, Israel.
SOURCE: JOURNAL OF IMMUNOLOGY, (1998 Oct 15) 161 (8) 3943-9. Journal code: IFB. ISSN: 0022-1767.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals; Cancer Journals
ENTRY MONTH: 199901
ENTRY WEEK: 19990104

AB Previous studies with CTL lines and CTL hybridomas have suggested that functional CD95 (APO-1/Fas)-**ligand** (CD95L) expression on effector CTLs is a consequence of specific CTL-target recognition and TCR triggering of newly transcribed CD95L. Such a control on the expression of CD95L could provide a double safeguard for killing only cognate target cells. Here the regulation of CD95L expression and function was tested in in vivo primed, alloreactive peritoneal exudate CTL (PEL) from perforin-deficient (P0) mice. CD95L-based, PEL-mediated cytotoxicity was blocked by brefeldin A, an **inhibitor** of **intracellular protein** transport, but not by the protein synthesis **inhibitor** emetine, the immunosuppressive drug cyclosporin A, or the DNA transcription **inhibitor** actinomycin D. CD95L mRNA transcripts in freshly isolated PEL were shown by RT-PCR; CD95L surface expression was evident by staining with Fas-Fc as well as CD95L Abs. Undiminished CD95L expression and cytocidal activity were found in PEL incubated for 48 h in culture, without adding Ag, mitogen, or cytokines. PEL expressed functional CD95L, yet exhibited target cell-specific killing, except when encountering high CD95-expressing cells. The results indicate that PEL use CD95L probably expressed in the Golgi and/or on the cell surface and do not require newly transcribed CD95L upon target cell **conjugation**. Hence the TCR-triggered recruitment of preformed CD95L, rather than its biosynthesis, controls CD95L-based specific lysis induced by CTL.

Searcher : Shears 308-4994

L27 ANSWER 11 OF 29 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 1998169500 MEDLINE
DOCUMENT NUMBER: 98169500
TITLE: The cleaved peptide of the thrombin receptor is a strong platelet agonist.
AUTHOR: Furman M I; Liu L; Benoit S E; Becker R C; Barnard M R; Michelson A D
CORPORATE SOURCE: Center for Platelet Function Studies, University of Massachusetts Medical Center, Worcester, MA 01655, USA.. Mark.Furman@banyan.ummed.edu
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1998 Mar 17) 95 (6) 3082-7.
Journal code: PV3. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199806
ENTRY WEEK: 19980603
AB Thrombin cleaves its G-protein-**linked** seven-transmembrane domain receptor, thereby releasing a 41-aa peptide and generating a new amino terminus that acts as a tethered **ligand** for the receptor. Peptides corresponding to the new amino terminal end of the proteolyzed seven-transmembrane domain thrombin receptor [TR42-55, SFLLRNPNDKYEPF, also known as TRAP (thrombin receptor-activating peptide)], previously have been demonstrated to activate the receptor. In this study, we demonstrate that the 41-aa cleaved peptide, TR1-41 (MGPRRLLLVAACFSLCGPLLSARTRARRPESKATNATLDPR) is a strong platelet agonist. TR1-41 induces platelet aggregation. In whole-blood flow cytometric studies, TR1-41 was shown to be more potent than TR42-55 and almost as potent as thrombin, as determined by the degree of increase in: (i) platelet surface expression of P-selectin (reflecting alpha granule secretion); (ii) exposure of the fibrinogen binding site on the glycoprotein (GP) IIb-IIIa complex; and (iii) fibrinogen binding to the activated GPIIb-IIIa complex. As determined by experiments with **inhibitors** [prostaglandin I2, staurosporine, wortmannin, the endothelium-derived relaxing factor congener S-nitroso-N-acetylcysteine (SNAC), EDTA, EGTA, and genestein], and with Bernard-Soulier or Glanzmann's platelets, we demonstrated that TR1-41-induced platelet activation is: (i) inhibited by cyclic AMP; (ii) mediated by protein kinase C, phosphatidyl inositol-3-kinase, myosin light chain kinase, and **intracellular protein** tyrosine kinases; (iii) dependent on extracellular calcium; and (iv) independent of the GPIb-IX and GPIIb-IIIa complexes. TR1-41-induced platelet activation was synergistic with TR42-55. In summary, the cleaved peptide of the seven-transmembrane domain TR (TR1-41) is a strong platelet agonist.

Searcher : Shears 308-4994

L27 ANSWER 12 OF 29 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 1998322161 MEDLINE
DOCUMENT NUMBER: 98322161
TITLE: Role for tyrosine phosphorylation and Lyn tyrosine kinase in fas receptor-mediated apoptosis in eosinophils.
AUTHOR: Simon H U; Yousefi S; Dibbert B; Hebestreit H; Weber M; Branch D R; Blaser K; Levi-Schaffer F; Anderson G P
CORPORATE SOURCE: Swiss Institute of Allergy and Asthma Research (SIAF), University of Zurich, Davos, Switzerland.. hus@siaf.unizh.ch
SOURCE: BLOOD, (1998 Jul 15) 92 (2) 547-57.
Journal code: A8G. ISSN: 0006-4971.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals; Cancer Journals
ENTRY MONTH: 199810
AB Fas **ligand**/Fas receptor molecular interactions have been implicated as having an important function for the regulation of eosinophil apoptosis. The purpose of the present study was to investigate biochemical events triggered by the engagement of the Fas receptor in freshly isolated human and mouse eosinophils. Activation of the Fas receptor on eosinophils with the agonistic anti-Fas monoclonal antibody (MoAb) resulted in increased tyrosine phosphorylation of several **intracellular proteins** . The tyrosine kinase **inhibitors** lavendustin A and genistein inhibited Fas receptor-induced cell death in both human and mouse eosinophils in vitro and prevented, at least partially, Fas receptor-mediated resolution of eosinophilic inflammation in a mouse in vivo model of lung eosinophilia. In addition, in freshly purified human eosinophils, lavendustin A prevented anti-Fas MoAb-induced proteolytic cleavage of lamin B, suggesting that tyrosine kinases may amplify the proteolytic signaling cascade within interleukin-1beta converting enzyme (ICE) family proteases. Moreover, the tyrosine kinase Lyn was identified as being involved in Fas receptor-mediated cell death. Collectively, these results demonstrate that tyrosine phosphorylation is an important step in the generation of the Fas receptor-**linked** transmembrane death signal in eosinophils and that Lyn participates in this pathway.

L27 ANSWER 13 OF 29 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 1998117305 MEDLINE
DOCUMENT NUMBER: 98117305
TITLE: Colon carcinoma cells use different mechanisms to

escape CD95-mediated apoptosis.

AUTHOR: von Reyher U; Strater J; Kittstein W; Gschwendt M; Krammer P H; Moller P
CORPORATE SOURCE: Institute of Pathology, University of Ulm, Germany.
SOURCE: CANCER RESEARCH, (1998 Feb 1) 58 (3) 526-34.
Journal code: CNF. ISSN: 0008-5472.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199804

AB CD95(APO-1/Fas) is a cell surface receptor that, when oligomerized by natural **ligand**, CD95L, or antibody, confers an apoptotic signal to apoptosis-sensitive cells. Whereas CD95 is expressed in every colonocyte of normal colon mucosa, CD95 is down-regulated or lost in the majority of colon carcinomas. To investigate the sensitivity to CD95-mediated apoptosis of normal and neoplastic colonocytes, we applied cross-**linking** CD95(anti-APO-1) monoclonal antibody to freshly isolated colon crypts and colon carcinoma cell lines and monitored apoptosis by DNA fragmentation and morphology. Normal colonocytes were constitutively sensitive to CD95-mediated apoptosis. All carcinoma lines were constitutively resistant but were sensitized upon pretreatment with IFN-gamma. Transcription **blocking**, **protein** synthesis, and export in carcinoma cells indicated that even low surface levels of CD95 were sufficient to efficiently transmit the signal. Despite low CD95 surface levels of non-IFNgamma-treated cells, actinomycin D, cycloheximide, and brefeldin A each sensitized all cell lines, but at different rates and kinetics. In this context, it was observed that a greatly delayed apoptotic response of SW620 cells was associated with the absence of antibody-induced CD95 capping. Phorbol 12-myristate 13-acetate inhibited CD95-mediated apoptosis by counteracting the IFNgamma-, actinomycin D-, and cycloheximide-mediated but not the brefeldin A-mediated sensitization. This phorbol 12-myristate 13-acetate-induced protection against apoptosis was completely abolished by staurosporine and by a selective protein kinase C **inhibitor**, Goe 6983. We conclude that, during malignant transformation, colonocytes acquire different mechanisms to escape CD95-mediated apoptosis. These include abrogation of CD95, inhibition of CD95 capping, and activation of antiapoptotic programs, both governed by and independent of protein kinase C.

L27 ANSWER 14 OF 29 MEDLINE DUPLICATE 9
ACCESSION NUMBER: 1998106174 MEDLINE
DOCUMENT NUMBER: 98106174
TITLE: Identification of tropoelastin as a **ligand** for the 65-kD FK506-binding protein, FKBP65, in the secretory pathway.

Searcher : Shears 308-4994

AUTHOR: Davis E C; Broekelmann T J; Ozawa Y; Mecham R P
CORPORATE SOURCE: Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, Missouri 63110, USA.. davis16@utsw.swmed.edu
CONTRACT NUMBER: HL 41926 (NHLBI)
HL53325 (NHLBI)
AR 41474 (NIAMS)
SOURCE: JOURNAL OF CELL BIOLOGY, (1998 Jan 26) 140 (2) 295-303.
Journal code: HMV. ISSN: 0021-9525.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199804
AB The folding and trafficking of tropoelastin is thought to be mediated by intracellular chaperones, although the identity and role of any tropoelastin chaperone remain to be determined. To identify proteins that are associated with tropoelastin intracellularly, **bifunctional** chemical cross-**linkers** were used to covalently stabilize interactions between tropoelastin and associated proteins in the secretory pathway in intact fetal bovine auricular chondrocytes. Immunoprecipitation of tropoelastin from cell lysates after cross-**linking** and analysis by SDS-PAGE showed the presence of two proteins of approximately 74 kD (p74) and 78 kD (p78) that coimmunoprecipitated with tropoelastin. Microsequencing of peptide fragments from a cyanogen bromide digest of p78 identified this protein as BiP and sequence analysis identified p74 as the **peptidyl-prolyl** cis-trans **isomerase**, FKPB65. The appearance of BiP and FKBP65 in the immunoprecipitations could be enhanced by the addition of brefeldin A (BFA) and N-acetyl-leu-leu-norleucinal (ALLN) to the culture medium for the final 4 h of labeling. Tropoelastin accumulates in the fused ER/Golgi compartment in the presence of BFA if its degradation is inhibited by ALLN (Davis, E.C., and R.P. Mecham. 1996. J. Biol. Chem. 271:3787-3794). The use of BFA and other secretion-disrupting agents suggests that the association of tropoelastin with FKBP65 occurs in the ER. Results from this study provide the first identification of a **ligand** for an FKBP in the secretory pathway and suggest that the prolyl cis-trans isomerase activity of FKBP65 may be important for the proper folding of the proline-rich tropoelastin molecule before secretion.

L27 ANSWER 15 OF 29 MEDLINE
ACCESSION NUMBER: 96215091 MEDLINE
DOCUMENT NUMBER: 96215091
TITLE: Growth hormone (GH) and a GH antagonist promote GH receptor dimerization and internalization.
AUTHOR: Harding P A; Wang X; Okada S; Chen W Y; Wan W;

Kopchick J J
CORPORATE SOURCE: Edison Biotechnology Institute, Molecular and Cellular Biology Program, Ohio University, Athens, Ohio 45701-2979, USA.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 Mar 22) 271 (12) 6708-12.
Journal code: HIV. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199609
AB It has previously been shown that a human growth hormone (hGH) analog, hGH-G120R, acts as a GH antagonist (Chen, W. Y., Wight, D. C. , Wagner, T. E., and Kopchick, J. J. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 5061-5065; Chen, W. Y., White, M. E., Wagner, T. E., and Kopchick, J. J. (1991) Endocrinology 129, 1402-1408; Chen, W. Y., Chen, N-Y., Yun, J., Wang, X. Z., Wagner, T. E., and Kopchick, J. J. (1994) J. Biol. Chem. 269, 15892-15897). In this study, we report the ability of hGH and hGH-G120R to be internalized by GH receptor expressing cells. Additionally, results of chemical cross-**linking** experiments revealed that both native hGH and hGH-G120R form complexes similar in size to that expected for hGH when bound to recombinant hGH-binding protein (bp). The molecular mass of the complex was determined to be approximately 280 kDa which is consistent with multiple receptors interacting with the **ligand**. The predominant radiolabeled band detected was a complex of approximately 140 kDa which probably represents one GH molecule bound to one GH receptor. The cross-**linked** complexes were not detected in the presence of excess unlabeled hGH or hGH-G120R and were not observed in cells which do not express detectable levels of GH receptors. Also, GH induced tyrosine phosphorylation of a complex of proteins of approximately 95 kDa in these cells whereas hGH-G120R did not. Thus, we have separated the hGH or hGH-G120R/GHR binding and internalization capabilities from the ability to stimulate tyrosine phosphorylation of **intracellular proteins**.

L27 ANSWER 16 OF 29 MEDLINE
ACCESSION NUMBER: 97064207 MEDLINE
DOCUMENT NUMBER: 97064207
TITLE: Mutational analysis of the **ligand**-binding domain of M-T2 protein, the tumor necrosis factor receptor homologue of myxoma virus.
AUTHOR: Schreiber M; McFadden G
CORPORATE SOURCE: Department of Biochemistry, University of Alberta, Edmonton, Canada.
SOURCE: JOURNAL OF IMMUNOLOGY, (1996 Nov 15) 157 (10) 4486-95.

Journal code: IFB. ISSN: 0022-1767.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals; Cancer Journals
ENTRY MONTH: 199703
ENTRY WEEK: 19970301

AB The myxoma virus-encoded M-T2 protein shares extensive sequence homology with the **ligand**-binding domains of the TNF receptors (TNFRs) and has been shown to bind and inhibit rabbit TNF-alpha with affinities similar to those of TNF-alpha with cellular receptors. Here we show that M-T2 protein is secreted from infected cells as an N-**linked** glycoprotein, with both complex and hybrid or high mannose oligosaccharide chains. Since amino acid homology between M-T2 and cellular TNF receptors is limited to the four N-terminal cysteine-rich domains (CRDs), various M-T2 C-terminal truncations were created in recombinant vaccinia virus vectors. C-terminal deletions that include truncations up to the middle of the fourth CRD effectively bound and inhibited rabbit TNF-alpha. In contrast, removal of any one of the first three CRDs resulted in a mutant M-T2 protein incapable of binding or inhibiting rabbit TNF-alpha. The C-terminal portion of M-T2, which is not homologous to the cellular TNFRs, appears to be important for efficient secretion of M-T2 from infected cells, since all the C-terminal truncations, including a truncation removing only the last 24 amino acids, were effectively retained as **intracellular proteins** that were still capable of binding and inhibiting rabbit TNF-alpha. We conclude that the first three CRDs of M-T2 fulfill the same **ligand**-binding function as the cellular TNFRs, and the nonhomologous C-terminal region participates in protein trafficking of M-T2 in virus-infected cells.

L27 ANSWER 17 OF 29 MEDLINE DUPLICATE 10
ACCESSION NUMBER: 96249406 MEDLINE
DOCUMENT NUMBER: 96249406
TITLE: **Bifunctional** protein cross-linking reagents improve labeling of cytoskeletal proteins for qualitative and quantitative fluorescence microscopy.
AUTHOR: Safiejko-Mroczka B; Bell P B Jr
CORPORATE SOURCE: Department of Zoology, The University of Oklahoma, Norman, USA.
SOURCE: JOURNAL OF HISTOCHEMISTRY AND CYTOCHEMISTRY, (1996 Jun) 44 (6) 641-56.
Journal code: IDZ. ISSN: 0022-1554.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199610

AB Because permeabilization of the cell membrane is necessary to label **intracellular proteins** with most fluorescent probes, it is important to optimize the preservation and labeling of the proteins under study. We used qualitative and quantitative fluorescence microscopy to evaluate the effects of six different **bifunctional** protein cross-linking reagents and several extraction conditions on the labeling of filamentous actin with phalloidin and the immunolabeling of tubulin and gelsolin. The labeling of cytoskeletal and associated proteins can be significantly enhanced by the appropriate combination of **bifunctional** protein cross-linking reagents and extraction conditions. However, the conditions that give the most intense labeling vary depending on the label used. The greatest intensity of labeling with either phalloidin or antibodies was obtained with the intermediate-length cross-linker DSP. The two-step procedure of cross-linking with DSP and extracting in Triton X-100 in microtubule-stabilizing buffer containing DSP gives maximal labeling with phalloidin. Maximal labeling of gelsolin and tubulin with antibodies is obtained by extracting DSP-cross-linked cells with Triton in Hank's saline containing DSP. Therefore, DSP reproducibly improves preservation of both soluble and filamentous proteins for quantitative and qualitative studies by fluorescence microscopy.

L27 ANSWER 18 OF 29 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 96:31384 SCISEARCH
THE GENUINE ARTICLE: TM171
TITLE: CD38 SIGNAL-TRANSDUCTION IN HUMAN B-CELL PRECURSORS - RAPID INDUCTION OF TYROSINE PHOSPHORYLATION, ACTIVATION OF SYK TYROSINE KINASE, AND PHOSPHORYLATION OF PHOSPHOLIPASE C-GAMMA AND PHOSPHATIDYLINOSITOL 3-KINASE
AUTHOR: SILVENNOINEN O; NISHIGAKI H; KITANAKA A; KUMAGAI M A; ITO C; MALAVASI F; LIN Q; CONLEY M E; CAMPANA D (Reprint)
CORPORATE SOURCE: ST JUDE CHILDRENS HOSP, DEPT HEMATOL ONCOL, 332 N LAUDERDALE, MEMPHIS, TN, 38105 (Reprint); ST JUDE CHILDRENS HOSP, DEPT HEMATOL ONCOL, MEMPHIS, TN, 38105; HELSINKI UNIV, DEPT VIROL, HELSINKI, FINLAND; ST JUDE CHILDRENS HOSP, DEPT IMMUNOL, MEMPHIS, TN, 38105; UNIV TURIN, TURIN, ITALY; CNR, CIOS, TURIN, ITALY; UNIV TENNESSEE, COLL MED, MEMPHIS, TN, 38163
COUNTRY OF AUTHOR: USA; FINLAND; ITALY
SOURCE: JOURNAL OF IMMUNOLOGY, (01 JAN 1996) Vol. 156, No. 1, pp. 100-107.
ISSN: 0022-1767.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE

LANGUAGE: ENGLISH
REFERENCE COUNT: 56
*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Ligation of CD38 inhibits proliferation and induces apoptosis of human immature B cells, but the molecular mechanisms underlying this function are unknown. We found that CD38 dimerization with the specific mAbs T16 and IB4 induces rapid and transient tyrosine phosphorylation of several **intracellular proteins** in the immature B cell lines RS4;11, REH, 380, NaIm6, and OP-1. This effect could be markedly reduced by incubating cells with the tyrosine kinase **inhibitors** genistein, staurosporine, and herbimycin A. CD38 dimerization induced tyrosine phosphorylation of the protein kinase syk and increased syk kinase activity. CD38 dimerization also induced tyrosine phosphorylation of phospholipase C-gamma and of the p85 subunit of phosphatidylinositol 3-kinase (PI 3-K). The latter was accompanied by a distinct increase in PI 3-kinase activity in the immunoprecipitates obtained with an anti-phosphotyrosine Ab. In contrast to the signaling triggered by surface Ig engagement in B lymphocytes, CD38 ligation did not appear to induce tyrosine phosphorylation of the src-like protein tyrosine kinases lyn, fyn, and btk, or of vav- and ras-GTPase-activating protein, nor did it induce detectable changes in cytosolic Ca2+ concentrations. CD38 signaling also differed from cytokine-induced signaling in that it did not cause tyrosine phosphorylation of Jak1 and Jak2. Finally, CD38 ligation did not inhibit IL-3-induced tyrosine phosphorylation of Jak2. These results identify CD38 as a cell surface receptor with signal transduction properties activated by dimerization. Induction of signal transduction by CD38 ligation implies the existence of a yet unidentified natural **ligand** of CD38.

L27 ANSWER 19 OF 29 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 11
ACCESSION NUMBER: 1996:367523 BIOSIS
DOCUMENT NUMBER: PREV199699089879
TITLE: Catalytic lectin (leczyme) from bullfrog (Rana catesbeiana) eggs: Mechanism of tumoricidal activity.
AUTHOR(S): Nitta, Kazuo (1); Ozaki, Kouichi; Tsukamoto, Yoshimasa; Hosono, Masahiro; Ogawa-Konno, Yukiko; Kawauchi, Hiroaki; Takayanagi, Yoshio; Tsuiki, Shigeru; Hakomori, Sen-Itiroh
CORPORATE SOURCE: (1) Cancer Res. Inst., Tohoku Coll. Pharmaceutical Sciences, Komatsushima 4-4-1, Aoba-ku, Sendai 981 Japan
SOURCE: International Journal of Oncology, (1996) Vol. 9, No. 1, pp. 19-23.
ISSN: 1019-6439.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Catalytic lectins (leczymes) of frog eggs are sialic acid-binding

lectins that have intrinsic RNase activity. They inhibit tumor cell proliferation in vitro and in vivo, although their cytotoxic mechanism remains unclear. RNase A has no tumoricidal activity. It is hypothesized that leczymes bind to cell surface sialo-lycoconjugate receptors, enter the cell, and subsequently degrade RNA. In order to investigate the cytotoxic mechanism of cSBL, a leczyme from Rana catesbeiana egos, we established cSBL-resistant clone RC-150 from mouse leukemia P388 cells. cSBL-treated P388 cells showed extensive RNA degradation over the course of 1 h, whereas cSBL-treated RC150 cells showed no RNA degradation even over the course of 24 h. Treatment of P388 cells with cSBL led to decreased concentration of intracellular Ca-2+, decreased protein kinase A activity, and increased protein kinase G activity. Incubation with cSBL decreased glutathione levels and enhanced -lutathioneS-transferase (GST) activity in P388 cells, but had no effect on RC-150 cells. We conclude that cSBL-specific degradation of RNA occurs in cSBL-sensitive tumor cells, that cSBL leads to alteration of signal transduction and an **intracellular protein** kinase cascade reaction, and that internalized cSBL is detoxified by GST or thioltransferase. Our findings support a **bifunctional** model in which a leczyme is both an adhesive protein (binding to sialoglycoconjugates) and an enzyme (displaying RNase activity).

L27 ANSWER 20 OF 29 MEDLINE DUPLICATE 12
ACCESSION NUMBER: 94043346 MEDLINE
DOCUMENT NUMBER: 94043346
TITLE: Cytotoxicity of folate-Pseudomonas exotoxin **conjugates** toward tumor cells. Contribution of translocation domain.
AUTHOR: Leamon C P; Pastan I; Low P S
CORPORATE SOURCE: Department of Chemistry, Purdue University, West Lafayette, Indiana 47907-1393.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1993 Nov 25) 268 (33) 24847-54.
Journal code: HIV. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199402
AB Folate-protein **conjugates** can be nondestructively delivered into a cell's cytoplasm via folate receptor-mediated endocytosis if (i) the target cells express a folate-binding protein, and (ii) if the folate is **linked** to its attached protein at a site that does not interfere with receptor recognition. Because such **conjugates** have been observed to remain in endosomal compartments for extended periods following cellular uptake, we decided to evaluate whether release into the cytoplasm

might be expedited by inclusion of a translocation domain in the folate-protein construct. To test this possibility, momordin-folate and truncated Pseudomonas exotoxin-folate **conjugates** (LysPE38 and Cys-PE35), i.e. protein synthesis **inhibitors** either lacking or containing the desired translocation domain, respectively, were examined for their abilities to **block protein** synthesis in a variety of cell types. The translocation competent LysPE38-folate construct was found to kill cells six times more rapidly with 10-fold greater potency than the permeation-incompetent mormordin-folate. Further, cells expressing low levels of folate receptors could only be exterminated by the translocation competent Pseudomonas exotoxin-folate **conjugates**. When the translocation capability of CysPE35-folate was inactivated by modification of Cys287, the construct also lost most of its cytotoxicity. These data suggest that autocatalysis of transport from an internal vesicular compartment into the cytoplasm can greatly augment the cytotoxicity of a protein toxin entering cells via the folate endocytosis pathway. Because the folate **ligand** can selectively target a protein **conjugate** to cancer cells in the presence of normal cells, such translocatable toxin-folate constructs warrant further study as a possible treatment for some malignancies.

L27 ANSWER 21 OF 29 MEDLINE DUPLICATE 13
ACCESSION NUMBER: 93286054 MEDLINE
DOCUMENT NUMBER: 93286054
TITLE: ATPase-promoting dead end inhibitors of the cAMP-dependent protein kinase.
AUTHOR: Mendelow M; Prorok M; Salerno A; Lawrence D S
CORPORATE SOURCE: Department of Chemistry, State University of New York, Buffalo 14214.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1993 Jun 15) 268 (17) 12289-96.
Journal code: HIV. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199309

AB The cAMP-dependent protein kinase is a **bifunctional** enzyme, catalyzing the phosphorylation of the serine and threonine residues in peptides and proteins (kinase activity) as well as the phosphorylation of water (ATPase activity). We have found that several peptides, which serve as inhibitors of the kinase reaction, will either maintain or enhance the ATPase reaction catalyzed by the enzyme. Positively charged dipeptides (e.g. Arg-Arg), as well as small guanidino-containing compounds (e.g. guanethidine) **block protein** kinase activity yet enhance ATPase activity up to 3.5-fold over that exhibited by the enzyme in the

absence of these compounds. In contrast, several nonphosphorylatable peptides, whose primary sequences are based on that of a known substrate (i.e. Leu-Arg-Arg-Ala-Ser-Leu-Gly), such as Leu-Arg-Arg-Ala-Ala-Leu-Gly, Leu-Arg-Arg-Ala-Phe-Leu-Gly, and Leu-Arg-Arg-Ala-Tyr-Leu-Gly, have little or no effect on the rate of the kinase-catalyzed hydrolysis of ATP. An exception to the latter observation is Leu-Arg-Arg-Ala-Cys-Leu-Gly, a cysteine-containing peptide that promotes the protein kinase-catalyzed ATPase reaction by 2.2-fold. We have also found that peptides that possess relatively large amino acid side chain moieties immediately following the arginine dyad (i.e. such as Phe, Tyr, Cys, or Asn at Xaa in Leu-Arg-Arg-Xaa-Ala-Leu-Gly) sharply reduce the rate of enzyme-catalyzed ATP hydrolysis. This suggests that in the presence of peptides containing an -Arg-Arg-Ala- sequence, the enzyme-bound gamma-phosphate of ATP is relatively accessible to water. In contrast, when the latter alanine moiety is replaced by a larger residue, access by water to ATP appears to be hindered. These results indicate that certain structural features associated with the substrate or substrate analog have a profound influence on the manner by which these species interact with the protein kinase. Furthermore, the work described herein demonstrates that it is possible to block the physiologically important kinase reaction and simultaneously promote the energetically wasteful ATPase reaction.

L27 ANSWER 22 OF 29 MEDLINE DUPLICATE 14
ACCESSION NUMBER: 93139528 MEDLINE
DOCUMENT NUMBER: 93139528
TITLE: Cross-**linking** of CD14 molecules on monocytes results in a CD11/CD18- and ICAM-1-dependent adherence to cytokine-stimulated human endothelial cells.
AUTHOR: Beekhuizen H; Blokland I; van Furth R
CORPORATE SOURCE: Department of Infectious Diseases, University Hospital, Leiden, The Netherlands.
SOURCE: JOURNAL OF IMMUNOLOGY, (1993 Feb 1) 150 (3) 950-9. Journal code: IFB. ISSN: 0022-1767.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals; Cancer Journals
ENTRY MONTH: 199304
AB The myeloid differentiation protein CD14 that is expressed on the surface of mature monocytes contributes to the adherence of monocytes to cytokine-stimulated monolayers of human macrovascular endothelial cells (EC). It has also been observed that the initial adherence of monocytes to cultured cytokine-stimulated EC eventually results in an ICAM-1- and LFA-1 (CD11a/CD18)-dependent adherence, which coincides with stretching and lateral migration of the

monocytes over the surface of EC. Recently, it was reported that CD14 mediates monocyte activation and can induce a change in the avidity of CD11a/CD18 for its **ligand** ICAM-1. The aim of the present study was to investigate whether activation of monocytes by CD14 elicits a CD11/CD18-dependent adhesion of monocytes to ICAM-1 on rIL-1 alpha-stimulated EC. Incubation of monocytes with murine anti-CD14 mAb alone did not mobilize intracellular calcium but the subsequent addition of F(ab')2 anti-mouse Ig, which caused cross-**linking** of CD14 on the surface of monocytes, induced a transient rise in cytosolic free calcium concentration and enhanced the percentage monocytes that adhered to monolayers of macrovascular venous EC stimulated with rIL-1 alpha for 24 h, but not to nonstimulated EC. The elevated adhesion was decreased with monocytes were preincubated with staurosporine, an **inhibitor** of **intracellular protein** kinase activity and was markedly inhibited by mAb against the common beta 2-subunit (CD18) of the CD11/CD18 molecules on monocytes and by mAb against ICAM-1 on 24-h rIL-1 alpha-stimulated venous EC. These studies provide evidence for the hypothesis that the binding of monocytes via CD14 to rIL-1 alpha-stimulated EC generates an intracellular response in monocytes and triggers an adhesion mechanism that allows CD11/CD18 molecules on monocytes to bind to ICAM-1 on EC.

L27 ANSWER 23 OF 29 MEDLINE DUPLICATE 15
ACCESSION NUMBER: 89008604 MEDLINE
DOCUMENT NUMBER: 89008604
TITLE: Abnormal proteins as the trigger for the induction of stress responses: heat, diamide, and sodium arsenite.
AUTHOR: Lee K J; Hahn G M
CORPORATE SOURCE: Department of Radiation Oncology, Stanford University, California 94305-5468.
CONTRACT NUMBER: CA-04542 (NCI)
CA-32827 (NCI)
SOURCE: JOURNAL OF CELLULAR PHYSIOLOGY, (1988 Sep) 136 (3) 411-20.
Journal code: HNB. ISSN: 0021-9541.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 198901
AB Thermotolerance and synthesis of heat shock proteins are induced in cells in response to a variety of environmental stresses. We examined the suggestion of Hightower (1980) that modifications of **intracellular proteins** may be the triggering event that induces heat shock protein synthesis and thermotolerance. We did so by modifying cellular proteins, using diamide, a sulfhydryl oxidizing agent, and dithio-bis (succinimidyl propionate), an agent that cross-links **bifunctional** amino groups. Both of these

agents induced heat shock proteins and thermotolerance in CHO (HA-1) cells. Furthermore, we observed cross-resistance and self-tolerance with three seemingly unrelated stimuli (diamide, heat, and sodium arsenite). This observation suggests that the induction of protective responses to these stimuli is mediated by a common mechanism. The results support the hypothesis that production of abnormal proteins by various stresses induces the stress responses as well as tolerance.

L27 ANSWER 24 OF 29 MEDLINE DUPLICATE 16
ACCESSION NUMBER: 88254804 MEDLINE
DOCUMENT NUMBER: 88254804
TITLE: Cross-linking of alpha and gamma-thrombin to distinct binding sites on human platelets.
AUTHOR: Jandrot-Perrus M; Didry D; Guillin M C; Nurden A T
CORPORATE SOURCE: Laboratoire de Recherche sur l'Hemostase et la Thrombose, Faculte Xavier Bichat, Paris, France..
SOURCE: EUROPEAN JOURNAL OF BIOCHEMISTRY, (1988 Jun 1) 174 (2) 359-67.
Journal code: EMZ. ISSN: 0014-2956.
PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 198810

AB The interaction of thrombin with proteins at the platelet surface was assessed by chemical cross-linking with the membrane-impermeable reagents bis(sulphosuccinimidyl)suberate and dithiobis(sulphosuccinimidyl propionate) under conditions which induced no modification of **intracellular proteins** and minimal cross-linking of membrane glycoproteins. The proteins covalently linked to 125I-labelled alpha and gamma-thrombin were analyzed by sodium dodecyl sulfate/polyacrylamide gel electrophoresis and crossed immunoelectrophoresis. 125I-alpha-thrombin was detected in high-molecular-mass complexes (a) at the top of a 3% acrylamide stacking gel and (b) with a Mr approximately equal to 400,000. In addition, two complexes of 240 kDa and 78 kDa were characterized. Hirudin prevented the formation of each of these complexes. The 78-kDa complex occurred spontaneously in the absence of **bifunctional** reagents, was only observed with active alpha-thrombin and was not dissociated by hirudin. Such characteristics are similar to those of a serpin serine-protease complex. The 240-kDa complex was formed with 0.8-100 nM alpha-thrombin, was observed after a short incubation time (30 s) and occurred with TosLysCH2Cl-inactivated alpha-thrombin. After analysis of Triton-X-100-soluble extracts of cross-linked platelets by crossed immunoelectrophoresis against a rabbit antiserum to platelets, two principal precipitates contained 125I-alpha-thrombin. These were a precipitate containing GPIIb-IIIa complexes and a

precipitate in the position of GPIb. Indirect immunoprecipitation of GPIb, using a murine monoclonal antibody, confirmed it to be the major platelet component in the 240-kDa complex. Significantly, 125I-gamma-thrombin, which activates platelets with a prolonged lag phase, failed to bind to GPIb and complexes in the 240-kDa and 78-kDa molecular mass range were not observed. We conclude that several binding sites for alpha-thrombin are present at the platelet surface, and that GPIb is one of them. The studies with gamma-thrombin suggest that binding to GPIb is not obligatory for platelet activation although it could be involved in an initial step of the platelet response.

L27 ANSWER 25 OF 29 MEDLINE DUPLICATE 17
ACCESSION NUMBER: 88060371 MEDLINE
DOCUMENT NUMBER: 88060371
TITLE: Formaldehyde sensitivity of a GFAP epitope, removed by extraction of the cytoskeleton with high salt.
AUTHOR: Bell P B Jr; Rundquist I; Svensson I; Collins V P
CORPORATE SOURCE: Department of Pathology, Linkoping University, Sweden..
SOURCE: JOURNAL OF HISTOCHEMISTRY AND CYTOCHEMISTRY, (1987 Dec) 35 (12) 1375-80.
Journal code: IDZ. ISSN: 0022-1554.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 198803
AB We have used cytofluorometry to examine the formaldehyde sensitivity of the binding of a monoclonal antibody (MAB) to its epitope on glial fibrillary acidic protein in human malignant glioma cells in culture. When acetone-extracted whole cells or cytoskeletons, made by extracting with Triton in stabilizing buffer (Tsb), are fixed with formaldehyde, binding of the MAB Tp-GFAP1 to GFAP is abolished or greatly reduced. Fixation with the **bifunctional** protein crosslinking reagent dithiobis (succinimidyl propionate) (DTSP) has the same negative effect as formaldehyde. If cytoskeletons are further extracted with Tsb containing 250 mM ammonium sulfate (Thsb), fixation with formaldehyde or DTSP has reduced or no effect on the binding of Tp-GFAP1. The data are consistent with the hypothesis that aldehyde sensitivity of Tp-GFAP1 is caused by the crosslinking of a second protein to GFAP that blocks the binding of the MAB to its epitope. This putative **blocking protein** is part of the Triton-insoluble cytoskeleton, but it begins to be solubilized in 50 mM ammonium sulfate and it is largely removed in 250 mM ammonium sulfate (Thsb). SDS-PAGE shows that extraction with Thsb also removes a large number of proteins from the cytoskeleton, one of which could be the **blocking protein**. A second antibody to GFAP, designated Tp-GFAP3, was

raised against cytoskeletons which had been fixed with DTSP and in which the epitope recognized by Tp-GFAP1 was presumably blocked. Tp-GFAP3 is not sensitive to fixation by either formaldehyde or DTSP.

L27 ANSWER 26 OF 29 MEDLINE DUPLICATE 18
ACCESSION NUMBER: 84002423 MEDLINE
DOCUMENT NUMBER: 84002423
TITLE: Interactions of cytostatic unsaturated ketonucleosides with sulfhydryl containing cell constituents.
AUTHOR: Halmos T; Cardon A; Antonakis K
SOURCE: CHEMICO-BIOLOGICAL INTERACTIONS, (1983 Aug 15) 46 (1) 11-29.
Journal code: CYV. ISSN: 0009-2797.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 198401
AB The cytostatic unsaturated ketonucleosides, 1, 2, 3 and 4 are highly reactive sulfhydryl blocking agents. Kinetics of their reactions with reduced glutathione (GSH) were measured and their reactivity was compared to that of N-ethylmaleimide (NEM), acrylonitrile and chloroacetamide. Their reaction products with N-acetyl-L-cysteine (AcCys) were prepared and characterized by chemical analysis and nuclear magnetic resonance (NMR) spectroscopy. Compounds 1, 2 and 3 gave Michael type 1:1 addition products. Compound 4 reacted with AcCys by a three step mechanism; the primary addition product 8 underwent an unusual elimination reaction giving the unsaturated compound 9, which yielded the addition product 10 with AcCys. In the reaction with GSH, compound 4 behaved like a **bifunctional** SH alkylating agent. Compounds 1, 2, 3 and 4 also reacted with protein thiols as shown by their ability to inhibit lactate dehydrogenase (LDH). Unsaturated ketonucleosides had diversified effect on L1210 leukemia cells. While the most potent cytostatics, compounds 1 and 3, reduced considerably the membrane surface SH level, they were without effect on soluble **intracellular protein** thiols. In contrast, nucleosides 2 and 4, less active than the former, only slightly affected the membrane surface sulfhydryls and considerably depleted the intracellular soluble protein thiols. Only slight differences were found between the reactions of the four nucleosides with non-protein SH (NPSH). The correlation found between in vivo biological activity and cell membrane impairment suggests that selective alkylation of certain key membrane thiols by unsaturated ketonucleosides might be an important event in their biological effect.

L27 ANSWER 27 OF 29 MEDLINE DUPLICATE 19

ACCESSION NUMBER: 81101044 MEDLINE
DOCUMENT NUMBER: 81101044
TITLE: Permeabilization of animal cells for kinetic studies of intracellular enzymes: in situ behavior of the glycolytic enzymes of erythrocytes.
AUTHOR: Aragon J J; Feliu J E; Frenkel R A; Sols A
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1980 Nov) 77 (11) 6324-8.
Journal code: PV3. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198105

AB Intracellular enzymes in erythrocytes can be made accessible for in situ kinetic studies by treating the cells with **bifunctional** reagents to crosslink proteins, thus creating a network that allows subsequent permeabilization by delipidation without escape of **intracellular proteins**. Dimethyl suberimidate, dimethyl 3,3'-dithiobispropionimidate, and toluene-2,4-diisocyanate have been used successfully as crosslinking reagents, and digitonin has been used for delipidation. In a systematic study of the in situ behavior of the 11 glycolytic enzymes of rat erythrocytes, it was observed that Km and Vmax values for the majority of the enzymes are essentially the same in situ as in vitro. Lactate dehydrogenase (L-lactate:NAD+ oxidoreductase, EC 1.1.1.27) is inhibited by excess of pyruvate as much in situ as in vitro. Hexokinase (ATP:D-hexose 6-phosphotransferase, EC 2.7.1.1) was allosterically inhibited by glucose 6-phosphate nearly as much in situ as in vitro but was not affected by 2,3-biphosphoglycerate. The allosteric properties of 6-phosphofructokinase (ATP:D-fructose 6-phosphate 1-phosphotransferase, EC 2.7.1.11), glyceraldehyde-phosphate dehydrogenase [D-glyceraldehyde-3-phosphate:NAD+ oxidoreductase (phosphorylating), EC 1.2.1.12], and pyruvate kinase (ATP: pyruvate 2-O-phosphotransferase, EC 2.7.1.40) in situ were qualitatively similar to those observed in vitro, but some important quantitative differences were noticed. Particularly striking was the much greater activity of phosphofructokinase in situ compared to that in vitro at physiological concentrations of effector metabolites.

L27 ANSWER 28 OF 29 MEDLINE DUPLICATE 20
ACCESSION NUMBER: 77018393 MEDLINE
DOCUMENT NUMBER: 77018393
TITLE: [**Intracellular protein** breakdown.
VII. Cathepsin L and H; two new proteinases from rat liver lysosomes].
Intrazellularer Proteinabbau. VII. Kathepsin L und H: Zwei neue Proteinasen aus Rattenleberlysosomen.

Searcher : Shears 308-4994

AUTHOR: Kirschke H; Langner J; Wiederanders B; Ansorge S; Bohley P; Broghammer U
SOURCE: ACTA BIOLOGICA ET MEDICA GERMANICA, (1976) 35 (3-4) 285-99.
Journal code: 0E6. ISSN: 0001-5318.
PUB. COUNTRY: GERMANY, EAST: German Democratic Republic
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: German
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197701
AB Some properties (molecular weight, pI, temperature stability, action of selected inhibitors, substrate specificity and pH-activity dependence) of two not yet known cathepsins from rat liver lysosomes are compared with the properties of the known cathepsin B1. Cathepsin L is a thiolproteinase, has a molecular weight of 23--24000 and a pI of 5,8--6,1. By disc electrophoresis and isoelectric focusing there appear several protein bands which all have enzymatic activity. Leupeptin behaves as a strong inhibitor. The pH-optimum for digestion of proteins is close to 5,0. Cathepsin L does not hydrolyse esters and splits synthetic low molecular substrates only to a low degree. Cathepsin L stored in presence of glutathion and EDTA in liquid nitrogen kept its activity for some months. Cathepsin H is an aminopeptidase as well as an endopeptidase. An enzyme with these **bifunctional** properties was detected up to now only in E. coli but not in animal cells. Cathepsin H is a thiol-enzyme with a molecular weight of 28000 and a pI of 7,1. Strong inhibitors are leucyl-chlormethan and SH-blocking substances. Leupeptin shows only a weak inhibitory effect to this enzyme compared to its action on cathepsins L and B1. The pH-optimum for hydrolysis of all substrates is 6.0. Cathepsin H splits proteins, amino acid derivatives and selected N-protected amino acid derivatives. Cathepsin H compared to cathepsin L and B1 is quite temperature stable.

L27 ANSWER 29 OF 29 MEDLINE DUPLICATE 21
ACCESSION NUMBER: 76089210 MEDLINE
DOCUMENT NUMBER: 76089210
TITLE: Inhibition of respiration and phosphorylation by some maleimide derivatives.
AUTHOR: Rotenberg Y S; Kelman G Y
SOURCE: BIOKHIMIIA, (1975 May-Jun) 40 (3) 489-96.
Journal code: A28. ISSN: 0006-307X.
PUB. COUNTRY: USSR
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: Russian
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197605
AB The inhibitory effect of maleimide and ten its N-substituted mono- and **bifunctional** derivatives was studied in isolated rat

liver mitochondria. It was found that all these compounds **bl cked protein** and non-protein SH-groups and were the potent inhibitors of the respiratory chain. In the inhibitory activity of unsubstituted maleimide its alkylating properties play the dominant role. The introduction of some substitutors in the maleimide molecule lends the properties of hydrophobic inhibitors to these derivatives. A high toxicity of all compound studied was shown in in vivo experiments.

FILE 'HOME' ENTERED AT 09:40:27 ON 16 MAR 2001

liver mitochondria. It was found that all these compounds **blocked protein** and non-protein SH-groups and were the potent inhibitors of the respiratory chain. In the inhibitory activity of unsubstituted maleimide its alkylating properties play the dominant role. The introduction of some substitutors in the maleimide molecule lends the properties of hydrophobic inhibitors to these derivatives. A high toxicity of all compound studied was shown in in vivo experiments.

FILE 'HOME' ENTERED AT 09:40:27 ON 16 MAR 2001